Chapter I: Introduction

Bangladesh, an agro-based country whose economy depends mostly on agriculture. In 2020–2021, the contribution of agriculture was 12.09% (Bangladesh Economic Review, 2022). Livestock plays an important role in nutrition directly through the consumption of animal products and by-products. In 2020–2021, livestock's contribution to Gross Domestic product (GDP) was 1.86% (Bangladesh Economic Review, 2022). The dairy sector is one of the most important parts of the livestock sector in Bangladesh. To meet the demand for milk and meat for the large population in Bangladesh, the number of dairy farms rearing high-yielding dairy cows is rising gradually. The number of cattle was 24.5 million, milk production was 9.46 million metric ton, and meat production was 7.09 million metric ton (Bangladesh Economic Review, 2022). To fulfill the extra demand, Bangladesh imports huge amounts of powder milk and dairy products. To fill up the deficit in milk and meat, almost 25% of the local demand is met by cattle from across the border (Yousuf *et.al.,* 2014). Under these circumstances, to meet the deficiencies in meat, milk, and milk products, the government and private organizations are putting efforts into enhancing the production of cattle farming in the country.

For meeting up the demand of deficiencies in meat, milk, and milk products the government sector, alongside many military farms, 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; and Gentech International, Grameen Motso O Pashusampad Foundation (GMPF), which are working on the dairy breed development program and providing technical assistance to the farmers (Herrero *et. al*. 2013). The number of registered medium- to large-sized farms as sources of milk and meat was 55,174 (Bangladesh Economic Review, 2022). All these farms are usually rearing cross bred cattle. Usually, Holstein-Frisian cross cattle are reared for optimum production of milk and meat on the farm. The expected level of milk and meat production is influenced by many factors, among which heat stress is one of the cardinal factors in the production of milk and meat in the farms. The reduction of the milk production is observed by the cardinal factors of the temperature and humidity of the cattle's shed and also cattle itself. Production and reproduction performance which is absolutely influenced by heat stress (Berian *et al,.*2019).

Heat stress happens when the body's way of controlling its internal temperature starts to fail. As well as air temperature, factors such as work rate, humidity and work clothing may lead to heat stress. Among the stressors, heat stress has been of major concern in reducing animal's productivity in tropical, sub-tropical and arid areas (Berian *et al,.*2019). The optimum environmental temperature for lactation depends on species, breed, and degree of tolerance to heat or cold. The milk yield of Holstein-cross cattle declines at temperatures above 21°C (Yousuf *et al.,* 2014). Heat stress affects the productive performance of dairy animals by reducing their dry matter intake (DMI), feed efficiency, and milk yield (Gantner *et al.,* 2011). Reduced feed intake during heat stress is the major reason for reduced milk production in dairy cows (Baumgard *et al.,* 2012). Changes in environmental variables such as ambient temperature, relative humidity, wind, and rainfall were recognized as potential hazards to livestock growth and production. Hematological parameters have been used to identify the effect of heat stress on productivity in dairy cattle (Grünwaldt *et al.,* 2005 and Berian *et al,.*2019). Blood parameter profiles as animal response indicators can serve as the basis for the diagnosis, treatment, and prognosis of diseases (Otto *et al*., 2000; Ndlovu *et al.,* 2007). Variations in haemato-biochemical parameters are found to be related to changes in ambient temperature and the temperature-humidity index, although within the physiological range for cattle.

Therefore, the seasonal variations may influence the haemato-biochemical profile of cattle (Otto *et al*., 2000; Ndlovu *et al.,* 2007). Productivity of dairy cattle depends on the use of specialized animals as well as on their reproductive health, nutritional characteristics, and environment in which they are raised (Yousuf *et al.,* 2014). Stress represents the reaction of body to stimuli that disturb normal physiological equilibrium or homeostasis, often with detrimental effects as shown by Khansari *et al* 1990. According to Stott (1981), stress is the result of environmental forces continuously acting upon animals which disrupt homeostasis resulting in new adaptations that can be detrimental or advantageous to the animal.

The degree to which an animal resists change in body temperature varies with different species because of differences in their heat regulating mechanisms. The physiological responses of dairy cattle to raised temperatures, allowing us to better evaluate their ability to adapt and to cope with environmental stress (Ma YL *et al*.,2010). Under thermal stress, a number of physiological and behavioral responses vary in intensity and duration in relation to the animal genetic make-up and environmental factors through the integration of many organs and systems viz. behavioral, endocrine, cardiorespiratory, and immune systems. Rosales (1994) defined stress as the cumulative detrimental effect of various factors on the health and performance of animals.

Therefore, the research work on the effect of heat stress on the production and blood profile of dairy cattle in intensively reared dairy farms was performed with the overall objectives of knowing the effect of heat stress on the production and to know the effect of heat stress on the haemato-biochemical parameters of dairy cattle in intensively reared dairy farms in Chattogram.

1) Specific Objectives:

   i) To determine the alteration of milk production due to heat stress in lactating cattle.
   ii) To ascertain the changes in milk component between different lactating cattle due to heat stress;
   iii) To evaluate the haemato-biochemical changes in lactating cattle due to heat stress,

Chapter II: Review of Literature

2.1. Dairy Production in Bangladesh

Globally, the livestock sector plays a significant role to generate income by the poor and marginal farmers (Herrero *et al.,* 2013). At the same time, because of the exponentially rising human population, there is an increasing demand for livestock products due to increasing incomes and urbanization, which has changed the dietary habits of humans towards a preference for animal protein sources (Thornton,.2015). Currently, the dairy sector supports nearly 150 million households across the world (Faye and Konuspayeva 2012). An evident rise in world milk production during the past three decades, from 522 million metric tons (MT) in 1987 to 843 MT in 2018, highlights the increasing demand for milk and milk products (Faye and Konuspayeva ., 2012). Climate change, in particular global warming, will affect the health and welfare of farm animals, both directly and indirectly (IPCC, 2007).

2.2. Problems of dairy industry

Environmental factors, such as temperature and light, exert significant effects on the production, health, and immunity of animals. Heat stress in tropical countries is a problem of great concern among farmers and livestock producers as it causes great economic loss in terms of both production and reproduction traits of animals. Crossbred cattle are more susceptible to physical distress when exposed to heat stress as compared to other farm animals. Under normal conditions, fluctuations in the body temperature of cows are very small. The normal body temperature ranges from 38.5°C to 39.5°C in calves, from 38.0°C to 39.5°C in heifers, and from 38.0°C to 39.0°C in adult cows (Ma YL *et al*.,2010). While an ambient temperature between 0°C and 25°C has been shown to be beneficial to production performance (McDowell *et al*.,1975), ambient temperatures in excess of 25°C have been shown to cause an increase in the body temperature of dairy cows, thereby increasing heat stress (Correa-Calderon *et al*.,2004).

2.3. Stresses in dairy cattle

A study by Robinson *et al.,* (1986) showed that heat production was 124.5 kcal/kg of BW0.75 when beef cattle were exposed to an ambient temperature of 20°C but was 141.8 kcal/kg of BW 0.75 when they were exposed to hotter conditions (35°C). This indicates that heat production increases with an increase in ambient temperature, resulting in a concomitant increase in the body temperature of the cow. However, body temperatures that exceed normal values are not ideal, and cows have been shown to decrease their feed intake and heat exchange capacity accordingly (Könyves *et al.,* 2017). As a result, this leads to lower milk production and reproductive indices (Kadzere *et al.,* 2002), as well as increased costs for the dairy industry in the summer months (Kadzere *et al.,* 2011).

2.4. Heat stress and its causes

Heat stress can be simply defined as a condition that occurs when an animal cannot dissipate an adequate quantity of heat, whether it is produced or absorbed by the body, to maintain both thermal balances. Under temperatures, relative humidity (RH), solar radiation (RAD), and wind speed (WS) that exceed their thermal comfort zone, dairy cows suffer from heat stress. Heat stress reduces milk production, reproductive performance, and profit (Yadab *et al.,*2021). Heat stress is one of the major concerns that affects the production potential of dairy cattle in almost every part of the world. Elevated temperature and humidity negatively affect feed intake, negatively affecting the reproductive potential, which ultimately decreases milk production.

2.5 Effect of Heat stress on intensively reared dairy cows

High-yielding cows are more susceptible to heat stress than low-yielding cows. Climate change, defined as the long-term imbalance of weather conditions like temperature, radiation, wind, and rainfall characteristics of a particular region, is likely to be one of the main challenges for mankind during the present century. The earth’s climate has warmed in the last century (0.74 ± 0.18 °C), with the 1990s and 2000s being the warmest on instrumental record (Intergovernmental Panel on Climate Change, 2007).

Seasonal and environmental changes can affect hematological values in domestic animals (Feldman *et al.,* 2002). A rise in environmental temperature decreases milk production in milk-producing dairy cattle. With the rise in population, Increased livestock productivity is associated with increased production diseases that reflect changes in the blood profile (Hewett, 1974). Changes in environmental variables such as ambient temperature, relative humidity, wind, and rainfall were recognized as potential hazards to livestock growth and production.

Hematological parameters have been used to identify the effect of heat stress on productivity in dairy cattle (Grünwaldt *et al.,* 2005). Blood parameter profiles as animal response indicators can serve as the basis for the diagnosis, treatment, and prognosis of diseases (Otto *et al.,* 2000; Ndlovu *et al.,* 2007). Stress-induced changes in immune function have been recorded in dairy cattle, with alterations to cell-mediated and humoral immunity having a significant impact on immune competence, which may render an animal more susceptible to infections (Carroll and Forsberg, 2007).

Heat stress in dairy cows is caused by a combination of environmental factors (temperature, relative humidity, solar radiation, and air movement) (Ghosh *et al.,* 2017). Heat stress occurs in animals when there is an imbalance between heat production within the body and its dissipation. Heat stress is the sum of external forces in a homoeothermic animal that act to shift body temperature from the resting state. Heat stress reduces feed intake, milk yield, growth rate, and reproductive performance (Patel *et al.,* 2017), which leads to major economic losses for dairy farmers, especially in tropical countries.

2.6 Effect of heat stress on physiology and productivity of dairy cattle

2.6.1 Effect of heat stress on physiology of dairy cattle

Exposure of dairy cattle to high-temperature environments stimulates thermoregulatory mechanisms and produces reductions in the rates of metabolism, feed intake, and productivity (Abdelatif and Alameen, 2012). In order to maintain homeothermy, an animal must be in thermal equilibrium with its environment, which includes radiation, air temperature, air movement, and humidity (Kadzere *et al.,* 2002).

Animals suffer from heat stress when any combination of environmental conditions causes the effective temperature of the environment to be higher than the animal's "thermoneutral" zone (Armstrong, 2019; Berian *et al.,* 1994). The productivity of dairy cattle depends on the use of specialized animals as well as on their reproductive health, nutritional characteristics, and the environment in which they are raised. Selection for milk production reduces the ability of the cow to withstand the stress caused by heat, thereby increasing susceptibility to heat stress and decreasing production and reproductive efficiency during the hotter months of the year (Vasconcelos and Demetrio, 2011). Breeds of Exotic origin suffer more from heat stress, in part due to their higher productivity, which reduces their threshold of thermal comfort (Silva *et al.,* 2002).

2.6.2 Effect of heat stress on productivity of dairy cattle

A large amount of metabolic heat is produced by digesting roughage, which increases the body temperature of cows. As ambient temperature increases in the summer months and as body temperature concomitantly increases, cows decrease their feed intake to mitigate heat stress (Ammer *et al*., 2018), thereby leading to a gradual decline in milk production and a change in milk content. It has been reported that the feed intake of dairy cows begins to decrease when the ambient temperature reaches 25°C and sharply decreases when the ambient temperature exceeds 30°C, after which the feed intake is approximately 20% to 40% lower than the normal intake (Hahn *et al.,* 1999). Thus, an observed decrease in feed intake is an important index of heat stress in dairy cows, where the combination of decreased feed intake and increased ambient temperature gradually results in lower milk yield (Xue *et al.,* 2010). If environmental factors can be actively monitored and management practices altered accordingly to alleviate heat stress in dairy cows, milk yield and quality can be improved by boosting feed intake (Fournel *et al.,* 2017).

Heat stress also has a serious impact on pregnancy in dairy cows (Ingraham *et al.,* 1974). A statistical analysis of pregnancies in 20,606 cows from seven farms showed that the pregnancy rates were 39.4% (THI72.0), 38.5% (72.0 to 73.9), 36.9% (74.0 to 75.9), 32.5% (76.0 to 77.9), and 31.6% (>78.0). This indicates that the pregnancy rate of dairy cows decreases in a Temperature Humidity Index (THI)-dependent way when THI is greater than 72.0, with a decrease in pregnancy rate of 1.03% per unit increase in THI (Lozano Domínguez *et al.,*2005).

2.7. Effect of heat stress on milk production of dairy cattle

Heat stress affects the productive performance of dairy animals by reducing their dry matter intake (DMI), feed efficiency, and milk yield (Gantner *et al.,* 2011). Reduced feed intake during heat stress is the major reason for reduced milk production in dairy cows (Baumgard *et al.,* 2012). The optimum environmental temperature for lactation depends on species, breed, and degree of tolerance to heat or cold. Reduced milk production is the first perceived consequence of heat stress. The milk yield of Holstein-cross cattle declines at temperatures above 21 °C. Moreover, a study that investigated seasonal effects on milk yield showed that the milk yield of Holstein cows decreased by 10% to 40% in summer in comparison to the milk yield in winter (Du Preez *et al.,*1990), further highlighting the influence of heat stress on milk production.

Per unit increase in (THI) beyond 72, 0.2 kg reduction in milk yield was recorded in dairy cows (West *et al.,* 2003; Ravagnolo et al.,2000). For each point increase in the value of THI beyond 69, milk production drops by 0.41 kg per cow per day in the Mediterranean climatic regime19,20. Further, every 1 °C increase in air temperature above the thermal neutral zone causes a 0.85 kg reduction in feed intake, which causes a 36% decline in milk production (West et al., 2003; Rhoads *et al.,* 2009). In addition, it was established that during severe heat stress conditions, the mammary gland uses a negative regulatory feedback mechanism to reduce milk production (Silanikove *et al.,* 2009; Baumgard *et al.,* 2012). Heat stress may also offset the genetic progress achieved in increasing milk yield.

2.8. Effect of heat stress on milk parameters of dairy cattle

Elevated core body temperature will reduce milk output and the percentages of milk protein, fat, solids, and lactose. High temperatures and low relative humidity are the critical parameters contributing to heat stress (Lozano Domínguez *et al*.,2005). Consumption of great quantities of food results in an increase in metabolic increments, which require efficient thermoregulatory mechanisms to maintain body temperature and physiological homeostasis. It is rather complicated to determine precisely the moment when the cow enters heat stress because the incidence of heat stress is influenced not only by energy balance but also by the quantity of water and the metabolism of sodium, potassium, and Chlorine (Kadzere *et al.,* 2002).

An inability of organism to adapt to a newly created situation leads to health disturbances, reductions in nutritive needs and the production of milk, changes in the chemical composition of milk, and reproductive disorders. It has been established that only 35% of the reduction in milk yield is due to decreased feed intake, and the remaining 65% is due to the direct physiological effect to decreased feed intake, and the remaining 65% is due to the direct physiological effect of heat stress (Rhoads et al., 2009). Decreased nutrient absorption, alteration in rumen function, and hormonal imbalance are other factors that contribute to reduced milk production during heat stress (Bernabucci *et al.,* 2010). Heat stress is one of the major concerns that affects the milk parameters of dairy cattle.

2.9. Heat stress and physiological changes of dairy cattle

Heat stress causes changes in homeostasis and has been quantified by the measurement of physiological variables such as body temperature, respiratory rate, and hormone concentrations. Despite the cited and well-known differences in breeds and reactions to heat stress (McManus *et al.,* 2009), there is still little information regarding the critical levels of these traits in crossbred cows. temperature, which is exaggerated by extensive production systems. In these cases, management interventions may be possible, but they are difficult and expensive to implement, particularly in poorly adapted cattle.

2.10. Heat stress and hematological parameters

Changes in environmental variables such as ambient temperature, relative humidity, wind, and rainfall were recognized as potential hazards in livestock growth and production. Hematological parameters have been used to identify the effect of heat stress on productivity in dairy cattle (Grünwaldt *et al.,* 2005). Blood parameter profiles as animal response indicators can serve as the basis for diagnosis, treatment, and prognosis of diseases (Otto *et al.,* 2000; Ndlovu *et al.,* 2007). Hematological parameters have been used as indicators of animal responses to changes in environmental conditions.

All hematological parameter values obtained in the dairy cows were within the physiological reference range for dairy cattle. However, it should be noted that mean values of RBC, Hct, and Hb were less than physiological mean values for Holstein dairy cattle (Lumsden *et al.,* 1980). These results were in accordance with previous studies, which reported that lower values of RBC, Hct, and Hb concentrations could be associated with a hemodilution effect due to increased water consumption for evaporative cooling during the hottest period. (Belic et al., 2010; Casella et al., 2013;

Ikuta *et al.,* 2010; Mazzullo *et al.,* 2014). Conversely, in another study, erythrocyte number, hematocrit value, and hemoglobin concentration were increased in cows exposed to high temperatures due to hemoconcentration (Koubková *et al.,* 2002).

The WBC mean value was higher than the normal reference mean value of leucocytes in dairy cattle. It was in accordance with a previous study, which reported that WBC values were higher in heat-stressed dairy cows (Mazzullo *et al.,* 2014). The mean values of neutrophils, lymphocytes, and monocytes (Morar *et al*., 2018) were higher than the normal mean values reported by Lumsden et al., 1980, for healthy Holstein cattle. Mazzullo *et al.,* (2014) found an increase in lymphocytes and a decrease in the number of neutrophils and monocytes in dairy cows exposed to high temperatures. Other authors reported that relative lymphocyte numbers decreased in the hottest period, whereas neutrophil and monocyte values increased (Koubková *et al.,* 2002).

2.11 Heat stress and biochemical parameters

Variations in biochemical parameters are found to be related to changes in ambient temperature and temperature-humidity index, although within the physiological range for cattle (Otto *et al.,* 2000; Ndlovu *et al.,* 2007). These results provide insights into the physiological responses of dairy cattle to raised temperatures, allowing us to better evaluate their ability to adapt and to cope with environmental stress.

Heat stress resulted in a nonsignificant decrease in total protein value. A significant difference in Albumin was observed under heat stress (Berian *et al.,* 2019). Glucose was observed to increase heat stress. A significant increase in AST (U/L) values was observed in heat-stressed dairy cows. Stress results in a non-significant increase in the ALT value in a heat-stressed condition. Heat Stress results in a significant increase in mineral profile, as seen in Berian et al., (2019). Biochemical parameters are found variation related to changes in ambient temperature, and temperature-humidity index, although within the physiological range for cows. Values of carbohydrate and protein level of stressed animals are changed due to heat stress. Values of Glucose (mg/dl) of stressed animals was in stressed animals was declined significantly (Prathap et al.,2017). Values of Total Protein (g/L) of stressed animals was declined insignificantly compared to reference value. Values of Albumin (g/L) of stressed animals were declined as significant ranges compared to reference value (Berian *et al.,* 2019).

Chapter III: Materials and methods

3.1 Study area

The present investigation was conducted at Military Farm Chattogram, which is located at Chattogram Cantonment in Chattogram district (Figure 1). This farm was selected based on well-organized management practices and regular data keeping records. For intensive monitoring, it was easy, and the farm authority agreed to provide data and samples as the research required. The farm is situated five kilometers away from the Cantonment Baizid gate. The farming system of the dairy unit is intensive type, and face in, face out both systems are adopted. Cross breed of Holstein Friesian (HF) cattle is kept for milk production. A balanced feeding system, vaccination, and deworming are maintained properly according to doctors of the farm. High yielding varieties of green gasses e.g., Napier, Para, German, Jamboo, Oats are cultivated for the cattle in the farm area.

3.2 Study period

The study was conducted for a period of three months; starting from March 2022 to May 2022.

3.3 Selection of cow

There were a total of 1750 cattle in the farm; among them 1500 are female and 200 are male. Among the 1500 females, 780 are milking cows, 320 are dry and pregnant cows, and 400 are heifers. A total of 90 cross breed HF dairy cows were selected for the study. They were in 3^{rd}, 4^{th} and 5^{th} parity, and each parity was consisting of thirty cows, and Body Condition Score (BCS) was the same. The cows were ensured with unique identification number by proper tagging. Parity wise production data, temperature and humidity of the ambient, shed, and rectal temperatures of each cow were recorded for three months, from March 2022 to May 2022.

3.4 Data Collection

3.4.1 Shed and environmental temperature

Shed and environmental temperature and humidity were recorded. Shed and environmental temperature were taken regularly during milking of cows by using a digital thermometer. Temperature was been recorded by keeping the thermometer inside the shed. An ambient temperature was recorded from the environment of the cattle yard area. Thermometer were placed at outside of the cattle yard and temperature and humidity were recorded regularly at milking time of cows.

3.4.2 Rectal temperature (RT)

The rectal temperature of selected cows was recorded using a digital thermometer by keeping the thermometer in contact with the rectal mucosa for about 2 minutes. Observations were recorded, on the basis of which the animals were selected in sheds and as per lactating group. The rectal temperature of the cows was taken weekly. Rectal temperature was recorded in two times at hot humid time of the day. Records of ninety cows was maintained in a register regularly. The records of rectal temperature of all ninety cows for ninety days were entered into excel sheet.

3.4.3 Milk production and parameters

Parity wise milk production data for ninety cows was recorded from 1^{st} march 2022 to 31^{st} may 2022 for 92 days. Morning and evening milk production of each cow were recorded then summed-up daily. The average milk production of each cow as per parity was calculated in excel sheets. Milk parameters like fat, SNF, and total solids (TS) were analyzed weekly with Garber method at the experimental area, as they have those facilities.

3.5. Milk analysis

3.5.1 Fat Test

3.5.1.1 Assay principle

Milk fat is separated from proteins by adding sulfuric acid. The separation is facilitated by using amyl alcohol and centrifugation. The fat content is read directly via a special calibrated butyrometer. Gerber developed specialized butyrometers (tubes), pipettes, and centrifuges.

3.5.1.2 Materials and reagent

1. Milk sample
2. Sulfuric acid
3. Amyl alcohol
4. Butyrometer
5. Pipettes, and Garber centrifuge.

3.5.1.3. Procedure

A butyrometer is filled up with adding 10 ml sulfuric acid gently. 10.75 ml milk added in the butyrometer gently. The butyrometer is shaking gently by adding 01 ml amyl alcohol. Centrifugation is done by Garber centrifuge machine for 3-5 minutes. Calibration is done and reading is done from butyrometer. Corrected Lactometer Reading (CLR)= Lactometer Reading + (Temperature – 20) x 0.20

3.5.2 SNF Test

3.5.2.1 Assay principle

Milk SNF is separated from proteins by adding sulfuric acid. The separation is facilitated by using amyl alcohol and centrifugation. The fat content is read directly via a special calibrated butyrometer. Gerber developed specialized butyrometers (tubes), pipettes, and centrifuges.

3.5.2.2 Materials and reagent

1. Milk sample
2. sulfuric acid
3. Amyl alcohol
4. Butyrometer

5. Pipettes, and Garber centrifuge.

3.5.2.3 Procedure

A butyrometer is filled up with adding 10 ml sulfuric acid gently. 10.75 ml milk added in the butyrometer gently. The butyrometer is shaking gently by adding 01 ml amyl alcohol. Centrifugation is done by Garber centrifuge machine for 3-5 minutes. Calibration is done and reading is done from butyrometer. Corrected Lactometer Reading (CLR)/4 + (0.22 x Fat %) + 0.72

3.6. Haemato-biochemical analysis

The blood sample was collected in the month of May for hematological analysis. The blood samples were taken from all experimental cows from experimental areas. The blood samples were shifted to Department of Physiology, Biochemistry, and Pharmacology, Chattogram Veterinary and Animal Sciences University (CVASU).

3.6.1. Collection and preservation of blood sample

Approximately 5 ml of blood were aseptically collected in the hottest time of the month of May from 1200 a.m. to 1400 p.m., from the jugular vein of cows by proper restraining techniques. Collected 2 ml blood samples were kept in vacutainers with EDTA anticoagulant and 3-ml in vacutainers without anticoagulant for smooth coagulation and serum separation. Collected blood samples were 144 in number and kept in a cool box for transportation from the study area to the research laboratory of the Department of Physiology, Biochemistry, and Pharmacology, Chattogram Veterinary and Animal Sciences University (CVASU). After reaching in laboratory, coagulated serum samples were centrifuged using the Vacutainer centrifuge machine at 3000 rpm for 15 minutes. The serum was separated and shifted to a vacutainer tube micropipette.

3.6.2 Hematological test of blood sample

Hematological parameters were performed using an auto hematology analyzer (Cell pack Alpha, Japan). All samples were analyzed for red blood cells (RBC), hematocrit (Hct), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentrations (MCHC), white blood cells (WBC), and platelet (Plt). The means of each hematological parameter were calculated and then compared with the average of reference range values for healthy Holstein cattle reported by Lumsden et al.,1980.

3.6.3 Preservation of serum samples

The obtained serum samples were stored at -20ºC until analysis for biochemical test.

3.6.4 Biochemical assay of serum sample

Proper aseptic measures were taken 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), Sodium (Na), Calcium (Ca), Phosphorus (P), Chloride (Cl) were assayed using an automated Biochemical analyzer (Humalyzer-3000, Germany) according to directions provided by the manufacturer of the kit. Randox kits were used to determine glucose, TP, Alb, Na; Ca;Cl; Chroma test kit for P; Biorex kit for AST and ALT. A total of 72 serum samples were analyzed as described in the blood sampling protocol.

3.6.4.1 Glucose Assay

3.6.4.1.1 Assay principle

The principal outcome of glucose is based on the principle of competitive binding between glucose in the test specimen and the GOD-POD reagent of glucose. The glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide reacts under the catalysis of peroxidase with phenol and 4 aminophenazone to a red-violet quinoneimine dye as an indicator. Colorimetric spectrophotometric methods were used for determination of glucose concentrations (Barham and Trinder,1972).

Reaction

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

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

3.6.4.1.2 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 gloves.

3.6.4.1.3 Procedure

The sterile eppendorf tubes were taken. 1000 µl of glucose conjugate reagent were taken into each eppendorf tube. Then 10 µl of glucose standard was added to the reagent in the eppendorf tube, and 10 µl of samples serum were taken in each eppendorf tube. The eppendorf tube was then incubated at 37°C for 10 minutes.

Glucose standards with conjugate reagents were examined first to determine of the standard value. Then all eppendorf tubes containing sample serum with glucose conjugate reagent were examined by a biochemical analyzer at 500 nm, and the readings were taken. The standard value was used as a comparison tool.

3.6.4.2 Total protein assay

3.6.4.2.1 Assay principle

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

3.6.4.2.2 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.

3.6.4.2.3 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 37°C 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.

3.6.4.3 Albumin assay

3.6.4.3.1 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.

3.6.4.3.2 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.

3.6.4.3.3 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 37°C 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.

3.6.4.4 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).

3.6.4.4.1 Calcium (Ca) assay

3.6.4.4.1.1 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.

3.6.4.4.1.2 Materials and reagents

1. Serum sample

2. Ca conjugate reagent

3. Precision pipettes: 25 µl, 500 ul

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

3.6.4.4.1.3 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 RI and 500 µl R2 of Ca conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37°C for 5 minutes. Ca standards with conjugatereagent 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 was used as a compared tool.

3.6.4.4.2 Sodium (Na)

3.6.4.4.2.1 Assay Principle

Sodium (Na) was estimated by Colorimetric method which is based on principle that potassium reacts with sodium tetra phenol boron in a specially prepared buffer to form a colloidal suspension. The amount of the turbidity produced is directly proportional to the concentration of potassium in the sample. The principles outcome of Sodium (Na) is based on the principle of competitive bindings between Na and Na reagent. Sodium combines with pottasium in an alkaline medium to form a red coloured complex. Interference of Sodium 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 Sodium present in the sample. Reaction: PH 11.5 Sodium + potassium Sodium - potassium complex

3.6.4.4.2.2 Materials and reagents

1. Serum sample

2. Na 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.

3.6.4.4.2.3 Procedure

The sterile eppendorf tubes were taken. Then 10 µl of Na standards was taken in an eppendorf tube and 10 µl of sample serums were taken in each eppendorf tube. 1000 µl of Na conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37°C for 5 minutes. Na standards with conjugate reagent wereexamined first for determination of the standard value. Then all eppendorf tubes containing sample serum with Na conjugate reagent was examined by automated humalyzer at 520 nm and the reading was taken. The standard value was used as a compared tool.

3.6.4.4.3 Phosphorus (P) assay

3.6.4.4.3.1 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: Pi PO_4^{3-} + H^+ + $(NH_4)_6Mo_7O_{24}$ phosphomolybdic complex

3.6.4.4.3.2 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.

3.6.4.4.3.3 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 37°C 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.

3.6.4.4.4 Chloride (Cl)

3.6.4.4.4.1 Assay Principle

Chloride (Cl) was estimated using Ferric Thiocyanate Method, which is based on principle when chloride is mixed with a solution of undissociated mercuric thiocyanate, the chloride preferentially combines with mercury forming mercuric chloride. The thiocyanate released combines with ferric ions present in the solution to form strongly colored ferric thiocyanate with an absorption maximum at 480 nm.

Reaction: PH 11.5 Mercury +Cloride mercuric - chloride complex

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.

3.6.4.4.4.2 Procedure

The sterile eppendrof tubes were taken. Then 10 µl of Cl standards was taken in an eppendrof tube and 10 µl of sample serums were taken in each eppendorf tube. 1000 µl of Cl conjugate reagent was then added to each eppendorf tube.The eppendorf tube was then incubated at 37°C for 5 minutes. Cl standards with conjugate reagent were examined first for determination of the standard value. Then all eppendorf tubes containing sample serum with Na conjugate reagent was examined by automated Humalyzer at 520 nm and the reading was taken. The standard value was used as a compared tool.

3.6.4.5 Alanine aminotransferase (ALT)

3.6.4.5.1 Assay Principle

Alanine aminotransferase (ALT) activities were determined by using standard kits from Trans Asia Bio-Medicals. The principle reaction of colorimetric determination of ALT activity is based on the reaction of aspartate or alanine with α-ketoglutarate to form oxaloacetate or pyruvate, respectively. The oxaloacetate or pyruvate formed was measured by monitoring the concentration of oxaloacetate or pyruvate hydrazine formed with 2, 4-dinitrophenyl hydrazine.

3.6.4.5.2 Procedure

The use of saline mix H_2O and perform a new Gain Calibration in the cell mode. Select in the Bun Test screen carry out water blink as instructed. Pipette with a test tube sample 0.05 ml and reagent 0.5 ml are mix and aspirate into the Rx Monza. Alanine aminotransferase (ALT) activities were determined by using standard kits from Transasia Bio-Medicals. The principle reaction of colorimetric determination of AST and ALT activity is based on the reaction of aspartate or alanine with α-ketoglutrate to form oxaloacetate or pyruvate, respectively. The oxaloacetate or pyruvate formed was measured by monitoring the concentration of oxaloacetate or pyruvate hydrazine formed with 2, 4-dinitrophenyl hydrazine.

Reaction:

α- oxoglutarate + l- alanine $\longrightarrow$ L- glutamate + pyruvate

Pyruvate + NADH + H^+ $\longrightarrow$ L- lactate + NAD^+

3.6.4.6 Aspartate aminotransferase (AST)

3.6.4.6.1 Assay Principle

Aspartate aminotransferase (AST) activities were determined by using standard kits from Trans Asia Bio-Medicals. The principle reaction of colorimetric determination of AST is based on the reaction of aspartate or alanine with α-ketoglutarate to form oxaloacetate or pyruvate, respectively. The oxaloacetate or pyruvate formed was measured by monitoring the concentration of oxaloacetate or pyruvate hydrazine formed with 2, 4-dinitrophenyl hydrazine.

3.6.4.6.2 Procedure

Kiratic method for the determination of GOT (ASAT) activity according to the recommendation of the Expert Panel of the IFCC (International Federation of the Clinical Chemistry). Without pyridoxal phosphate activation. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by using standard kits from Transasia Bio-Medicals.

The principle reaction of colorimetric determination of AST and ALT activity is based on the reaction of aspartate or alanine with α-ketoglutrate to form oxaloacetate or pyruvate, respectively. The oxaloacetate or pyruvate formed was measured by monitoring the concentration of oxaloacetate or pyruvate hydrazine formed with 2, 4-dinitrophenyl hydrazine.

Reaction:

$$\text{2- oxoglutarate} + \text{L- aspartate} \xrightleftharpoons{\text{GOT}} \text{L- glutamate} + \text{oxaloacetate}$$

$$\text{Oxoglutarate} + \text{NADH} + H^{+}: \xrightleftharpoons{\text{MOH}} \text{L- malate} + NAD^{+}$$

3.7 Statistical analysis

All recorded data were entered into MS-Excel 2011 or sorted out, coded then exported into STATA/IC 14.2 (Stata Corporation, USA). Descriptive statistics including percentage, mean standard deviation were performed. One way ANOVA was used to compare the continuous variables among groups of cows, months and lactation. A probability level of $p<0.05$ was considered to be significant.

Digital Thermometer

Temperature Collection

Sample Collection

Data Collection

Data and Sample Collection

Data and Sample Collection

Fig.1: Activities performed during the study

Selection of intensively reared dairy cattle farm

↓

Selection of 3^{rd},4^{th} and 5^{th} lactating cows (N=90)

↓

Collection of data for 90 days from 90 dairy cows

↓

Sample of blood collection in May 2022

↓

Total blood samples, N= 72

↓

Blood haematological analysis

↓

Blood biochemical analysis

↓

Data analysis

Fig.2: Schematic layout of the study

Chapter IV: Results

The study was conducted to observe the effect of heat stress on the production of lactating cows. Animal shed and rectal temperature were recorded in cows at different lactation. Milk production and milk contents like fat, SNF, and total solids were analyzed weekly with Garber method in the experimental area. The blood samples were taken from all experimental cows from experimental areas. The blood samples were collected in the month of May for hematological and biochemical analysis. The findings are being discussed in the subsequent paragraph.

4.1. Variation in rectal temperature of lactating cows concerning environmental temperature

Table. 1: Rectal temperature (°F) of lactating cows (N=90)

	Rectal Temperature (Mean ± SD)			
Lactation	**March**	**April**	**May**	**P Value**
3rd lactation	101.67±0.5	101.90±0.3	101.75±0.4	0.07
4th lactation	101.59± 0.5	101.88 ±0.3	101.91± 0.4	0.05
5th lactation	101.65 ± 0.5	101.99 ± 0.4	101.81 ±0.3	0.04

This study was conducted to observe the effect of heat stress on the production of lactating cows. Therefore, both animal shed and rectal temperature were recorded in cows at different lactation. Results are shown in Table 1 and 2. The rectal temperature of cows at 3rd (101.90 ±0.3 °F) and 5th (101.99 ±0.4°F) lactation increased in the month of April, whereas, increased rectal temperature (101.91 ±0.4 °F) of 4th lactation was observed in cows at May. There was significant difference of rectal temperature between different months of 4th and 5th lactating cows.

Table. 2: Average rectal temperature (°F) and environmental temperature in the studied farm (N=90)

Parameters	March	April	May	P-value
Average Rectal Temperature (Mean ± SD)	101.64± 0.5	101.71± 0.3	101.82± 0.4	0.00
Environmental temperature (Mean ± SD)	99.75± 0.6	99.99± 0.6	100.21± 0.4	0.47

Table 2 shows the increased average environmental (100.21± 0.4°F) and rectal (101.82± 0.4°F) temperatures in May. There was no significant(p>0.05) difference among the average rectal temperatures and environmental temperatures of cows at different months recorded during the study period.

4.2. Effect of heat stress on milk production and content

Table 3. Effect of heat stress on milk production

Milk production (in liter) (Mean ± SD)				
Stages of cows	**March**	**April**	**May**	**P Value**
3rd lactation	12.12± 3.53	10.96± 3.08	10.25± 4.27	0.140
4th lactation	12.83± 3.26	12.44± 3.85	11.10± 3.93	0.170
5th lactation	11.71± 2.86	11.40± 2.90	10.76± 2.74	0.420
Average Milk Production	12.22± 3.23	11.60 ±3.32	10.70± 3.68	0.010

Table 3 shows the highest milk production was in all cows at 3rd (12.12 ± 3.53 liter), 4th (12.83 ± 3.26 liter), and 5th (11.71 ± 2.86 liter) lactation during March. The monthly milk production variation among stages of lactating cows was not significant. The average milk production was in March 12.22 ± 3.23 liter, which decreased to 11.60 ± 3.32 liter in April and 10.70 ± 3.68 liter in May. However, average milk production decreased in April and May in all lactation groups and the variation was significant (P<0.05).

Table 4. Effect of heat stress on milk composition

Milk composition (Mean ± SD)				
Milk components	**March**	**April**	**May**	**P Value**
Fat%	3.69 ± 0.39	3.82 ± 0.19	3.99± 0.19	0.003
SNF%	9.46± 0.25	8.76 ±1.53	8.58± 0.19	0.012
Total Solid %	13.15 ±0.44	12.59 ± 1.60	12.57 ±0.27	0.133

The results of milk contents like fat%, SNF**%**, and total solids% which were analyzed weekly with Garber method in the experimental area shows in Table 4. The Mean ± SD value of Fat%, SNF, and total solids was 3.69 ± 0.39, 9.46 ± 0.25, and 13.15 ±0.44, respectively in March. Results showed that Fat% significantly increased in April (3.82 ± 0.19 %) and May (3.99 ± 0.19 %) due to heat stress. Whereas, due to heat stress SNF% significantly decreased in April (8.71 ±1.53 %) and May (8.58 ± 0.19 %). Total Solids% insignificantly decreased in April (12.59 ± 1.60 %) and May (12.57 ±.27 **%).**

4.3. Effect of heat stress on Hematological parameters of lactating cows

Table. 5 Hematological parameters of lactating cows (N= 72)

Parameter	**Stages of cows (Mean ±SD)**			**P-Value**
	3rd lactation	**4th lactation**	**5th lactation**	
RBC (10^4/ µL)	7.72 ± 1.05	7.54 ± 1.16	8.04± 1.76	0.551
WBC (10^3/µL)	9.33 ± 2.20	11.46 ± 4.31	11.50±3.03	0.040
Hb (g/dL)	10.23 ± 1.57	9.99 ± 1.43	10.31±1.04	0.764
HCT %	33.18 ± 5.91	33.41 ± 5.17	31.21±7.28	0.624
MCV (fL)	44.18± 3.82	43.41 ± 4.52	44.21±6.51	0.624
MCH (Pg)	13.13± 1.07	13.93 ± 3.20	13.18±2.04	0.395
MCHC (g/dL)	30.28± 0.70	30.35 ±0.62	29.91±0.71	0.267
PLT 10^3/ µL	317.67±111.67	343.57±132.35	337.13±188.58	0.740

Reference value: *WBC (10^3/µL) 4-12(8); RBC (10^4/ µL) 5 -10(8); Hb (g/dL) 9 -15(12); HCT% 25-35(30); MCV (fL) 40-48(44); MCH(Pg)12-13(13); MCHC(g/dL) 29-30(30); PLT (10^3/µL)130–330(250) adopted from Kaneko et al., 1997 and Yousuf et al., 2014*

Hematological parameters were observed in heat-stressed cows during May. Mean ±SD values of hematological parameters are given in Table 5. The values of RBC, Hb, MCV, MCH, MCHC and PLT of stressed cows among different lactation were insignificant (in the month of May. The highest value of WBC (11.50±3.03 X10^3/ µL), RBC (8.04 ± 1.76 X10^4/ µL) and Hb (10.31± 1.04 g/dL) were observed in cows at 5th lactation. The study also revealed the highest value of HCT (33.41 ± 5.172%), MCV (33.41 ± 4.515fL) MCH (13.93 ± 3.195 Pg) MCHC (30.35 ± .623 g/dL), and PLT (343.57±132.351 X10^3/ µL) in cows at 4th lactation. However, insignificant variation existed among the Mean ±SD values of all hematological parameters of lactating cows, except WBC, where the variation was significant among the lactating cows.

Table. 6 Frequency of hematological parameters of lactating cows (N= 72)

Parameter	Standard	Normal range	Higher	Lower
		n (%)	n (%)	n (%)
RBC (10^4/ µL)	5 -10 (8)	69 (96)	3 (4)	0 (0)
WBC (10^3/µL)	4-12 (8)	56 (78)	16 (22)	0 (0)
Hb (g/dL)	9 -15 (12)	65 (90)	0 (0)	7 (10)
HCT %	25-35 (30)	58 (81)	14 (19)	0
MCV (fL)	40-48(44)	54 (75)	9 (12.5)	9 (12.5)
MCH (Pg)	12-13 (13)	29 (40)	35 (49)	8 (11)
MCHC (g/dL)	29-30 (30)	29 (40)	41(57)	2 (3)
PLT 10^3/ µL	130-330 (250)	45 (63)	25 (34)	2 (3)

Hematological parameters of the 72 samples were observed in heat-stressed cows during May. The values of hematological parameters are given in Table 6. The values of WBC, RBC, Hb, HCT% and MCV was normal value range whereas MCH, MCHC and PLT was higher in value.

4.4. Effect of Heat Stress on Biochemical Parameters

Table 7. Blood biochemical parameters in lactating cows (N=72)

Parameters	**Stages of cows (Mean ± SD)**			**P-Value**
	3rd lactation	**4th lactation**	**5th lactation**	
Glucose (mg/dl)	25.05± 6.39	24.89 ± 7.52	20.725±12.64	0.263
Total Protein (g/L)	82.72 ± 11.45	84.727±13.44	85.915±19.52	0.75
Albumin (g/L)	39.8 ± 5.01	40.14 ±5.25	39.24±7.95	0.892
Na (mmol/L)	135.18±10.35	131.04 ±8.29	129.19±9.48	0.0988
P (mmol/L)	6.43 ± 1.03	6.46 ± 0.90	6.88±2.83	0.6295
Ca (mg/dl)	8.97± 0.99	8.73±1.44	8.55 ±1.28	0.4002
Cl (mmol/L)	119.51±12.74	121.59±8.43	120.98± 7.99	0.7355
ALT (U/L)	33.26 ±6.50	33.08 ±2.50	39 ±3.43	0.7132
AST (U/L)	68.36 ±9.80	73.82 ±6.58	89.125 ±18.84	0.0287

Reference value: *Glucose(mg/dl) 45-75(60); Total protein (g/L) 66- 87(75); Albumin(g/L) 38-51(45); Na (mmol/L) 125-155(140); P (mmol/L) 3.5 – 5.5 (4.5); Ca (mg/dl) 8-11(9.5); Cl (mmol/L) 98–120 (115); ALT (U/L) 16- 42 (30); AST (U/L) 37–120 (65) adopted from Kaneko et al., 1997 and Yousuf et al.,2014*

Different biochemical parameters were studied in May and variations in parameters were found in stressed cows at different lactation stages. Mean ±SD values of carbohydrate and protein levels of different lactating stressed animals are given in Table 7. The highest mean ±SD values of Glucose, Total Protein, and Albumin were (25.05± 6.39 mg/dl); (85.92±19.52 mg/dl); (40.14 ±5.25g/L), respectively, in 3rd, 5th and 4th lactating stressed cows in May. Mean ±SD values of Glucose, Total Protein, and Albumin found in stressed lactating cows were insignificant ($P>0.05$).

The mean ±SD values of minerals in heat-stressed cows are shown in Table 7. The highest mean ±SD values of Na, P, Ca, and Cl of stressed animals were 135.18±10.35 mmol/L, 6.88±2.83 mmol/L, 8.97±0.99 mg/dl, and 121.59±8.43 mmol/L, respectively of 3rd, 4th and 5th lactating cows in the month of May. In addition, no significant ($P>0.05$) variation among values of Na, P, Ca, and Cl was observed in milking cows at different lactation periods. Table 7 also shows the mean ±SD values of enzymes in heat-stressed lactating cows monitored in May. The highest mean ±SD values of blood ALT and AST of stressed animals were 39 ±3.43 U/L and 89.125 ±18.84 U/L, respectively of 5th lactating cows in May. This study also showed a significant ($P<0.05$) variation in blood AST levels among cows at different lactation periods.

Table 8. Comparison of blood biochemical parameters in lactating cows (N=72)

Parameter	Standard	Normal range	Higher	Lower
		n (%)	**n (%)**	**n (%)**
Glucose (mg/dl)	20-30(25)	49 (68.06)	14 (19.44)	9 (12.5)
Total Protein (g/L)	66- 87(75)	48 (66.67)	20 927.78)	4 (5.56)
Albumin (g/L)	38- 51(45)	43 (59.72)	2 (2.78)	27(37.5)
Na (mmol/L)	125-155(140)	64 (88.89)	0 (00)	8 (11.11)
P (mmol/L)	3.5 – 5.5 (4.5)	12 (16.67)	60 (83.33)	0 (00)
Ca (mg/dl)	8 -11(9.5)	56 (77.78)	4 (5.56)	12(16.67)
Cl (mmol/L)	98–120 (115)	44 (61.11)	28 (38.89)	0 (00)
ALT (U/L)	16- 42 (30)	61 84.72)	11 (15.28)	0 (00)
AST (U/L)	37–120 (65)	70 (97.22)	2 (2.78)	0 (00)

The biochemical parameters of the 72 samples were observed in heat-stressed cows during May. The values of biochemical parameters are given in Table 8. The values of Glucose, Total Protein, Albumin, Na, Ca, Cl, ALT and AST was normal valuer range whereas P value was higher.

Chapter V: Discussion

5.1 Effect of environmental temperature on cow physiology

Changes in environmental variables such as ambient temperature, relative humidity, wind and rainfall are recognized as potential hazards in livestock growth and production (IPCC, 2007). Heat stress affects the productive performance of dairy animals by reducing their dry matter intake (DMI), feed efficiency and milk yield (Armstrong *et al.*, 1994). Limited study on the heat stress effects of dairy cows' production, hematological and biochemical profiles exist in Bangladesh (Feldman *et al.*, 2002). Therefore, parameters regarding rectal and environmental temperatures, milk production and hematological and biochemical profiles had been used to study effect of heat stress on productivity in lactating cows (Berian *et al.*, 2019). In this study, the increased average environmental (100.21± 0.4°F) and rectal (101.82± 0.4°F) temperatures were observed due to heat stress in May (Kadzere *et al.*,2002). There was no significant ($p>0.05$) difference among the average rectal temperatures and environmental temperatures of cows at different months recorded during the study. This study corresponds well with the findings of (Abdelatif *et al.*, 2012).

5.1.1 Effect on body temperature

Heat production increases with an increase in ambient temperature, resulting in a concomitant increase in the body temperature of the cow. However, body temperatures that exceed normal values are not ideal, and cows have been shown to decrease their feed intake and heat exchange capacity accordingly which has been stated by Könyves et al., 2017. Heat stress leads to lower milk production and reproductive indices as well as increased costs for the dairy industry in the summer months (Kadzere et al., 2002, Kadzere et al., 2011). In Bangladesh, study on the heat stress which effects of dairy cows' production, haematological and biochemical profiles (Feldman *et al.*, 2002) was rare in intensively reared milking cows. Therefore, parameters regarding rectal and environmental temperatures, milk production and haematological and biochemical profiles had been used to study effect of heat stress on productivity in lactating cows (Berian *et al.*, 2019). In this study, the rectal temperature of cows at 3rd (101.90 ±0.3 °F) and 5th (101.99 ±0.4°F) lactation increased in the month of April (101.821 ± 0.364°F), whereas, increased rectal temperature (101.91 ±0.4 °F) was observed in cows at 4th lactation in May. There was significant ($p<0.05$) difference among the rectal temperatures of cows at 4th and 5th lactation which support with the findings of Silva *et al*., 2002.

5.2 Effect of environmental temperature and heat stress on milk

The productive performance of dairy animals is affected by heat stress which is defined by Rosales (1994) and heat stress has often with detrimental effects as shown by Khansari et al., 1990. According to Stott 1981. Milk production of 3rd lactating cows was in March 12.12± 3.53 which decreased in April 10.96± 3.08 and further decreased in May 10.25± 4.27. There was no significant variation ($p= 0.14$). Prathap *et al*. 2017 also found no variation in milk production affected by heat stress. Milk production insignificantly (p>0.0.05) decreased in April and May in all cows at different lactation periods during the study, which supports the findings of Abdelatif *et al.* (2012.

5.2.1. Association between milk production and heat stress

Collier et al.,(2009) shows that the value of the milk production was significantly changed with the heat stress which has similarity with the present study. The highest average milk production in all cows at 3rd (12.12 ± 3.53 liter), 4th (12.83 ± 3.26 liter), and 5th (11.71 ± 2.86 liter) lactation during March (Yousuf et al.,2014). The monthly milk productions variation among stages of lactating cows was not significant ($P>0.05$). The total average milk production was in March 12.22 ± 3.23 liter, which decreased to 11.60 ± 3.32 liter in April and 10.70 ± 3.68 liter in May (Khan, 2013 and Das *et al*., 2016). However, average milk production decreased in April and May and the variation was significant which is coincided with the earlier studies of Gantner et al., 2011 and Prathap et al.,2017 also.

5.2.2. Effect of heat stress on milk parameters

Yousuf *et al*., (2014) shows that the milk production and milk contents like fat%, SNF%, and total solids% were significantly changed with heat stress which present similar with the present study. The results of milk contents like fat, SNF, and total solids which were analyzed weekly with Garber method in the experimental area. The value of Fat%, SNF, and total solids was (3.69 ± 0.39 %), (9.46 ± 0.25 %), and (13.15 ±0.44 %), respectively in March (Abdelatif *et al.* 2012). Results showed that Fat% significantly (P<0.05) increased in April (3.82 ± 0.19 %) and May (3.99 ± 0.19 %) due to heat stress. Whereas, due to heat stress SNF% significantly decreased in April (8.71 ±1.53 %) and May (8.58 ± 0.19 %). Which supports the finding of Gantner et al., 2011 and Prathap et al.,2017 also.

5.2.3. Correlation between milk production and milk parameters

Berian *et al*., (2019) corresponds that the total average milk production significantly affected by heat stress which is similar with the present study. The present study shows that the total average milk production was in March 12.22 ± 3.23 liter, which decreased to 11.60 ± 3.32 liter in April and 10.70 ± 3.68 liter in May (Khan, 2013 and Das *et al*., 2016). The value of Fat%, SNF%, and total solids% was (3.69 ± 0.39 %), (9.46 ± 0.25 %), and (13.15 ±0.44 %), respectively in March (Abdelatif *et al.* 2012). Results showed that Fat% significantly (P<0.05) increased in April (3.82 ± 0.19 %) and May (3.99 ± 0.19 %) due to heat stress. Whereas, due to heat stress SNF% significantly decreased in April (8.71 ±1.53 %) and May (8.58 ± 0.19 %). (Kaneko *et al*., 1997 and Yousuf *et al*.,2014)

5.3. Effect of heat stress on Hematological parameters

Grünwaldt et al., (2005) stated that hematological parameters have been used to identify the effect of heat stress on productivity in dairy cattle. Blood parameter profiles as animal response indicators can serve as the basis for the diagnosis, treatment, and prognosis of diseases (Otto *et al*., 2000; Ndlovu *et al*., 2007). Hematological parameters have been used to identify effect of heat stress on productivity in dairy cows (Kadzere *et al*., 2002). The values of RBC, Hb, MCV, MCH, MCHC and PLT of stressed cows among different lactation were insignificant (P>0.05) in the month of May (Prathap *et al*., 2017).

The highest value of WBC (11.50±3.03 X10^3/ µL), RBC (8.04 ± 1.76 X10^4/ µL) and Hb (10.31± 1.04 g/dL) were observed in cows at 5^{th} lactation which is similar stated with Feldman *et al*., (2002). The study also revealed the highest value of HCT (33.41 ± 5.172%), MCV (33.41 ± 4.515fL) MCH (13.93 ± 3.195 Pg) MCHC (30.35 ± .623 g/dL), and PLT (343.57±132.351 $X10^3$/ µL) in cows at 4^{th} lactation (Yousuf *et al*.,2014). However, insignificant (P>0.05) variation existed among the values of all hematological parameters of lactating cows, except WBC, where the variation was significant (P<0.05) among the lactating cows (Berian *et al*., 2019).

Comparison of the hematological parameters of the samples were observed in heat-stressed cows during May. The values of WBC, RBC, Hb, HCT% and MCV was normal value range whereas MCH, MCHC and PLT was higher in value (Hewett, 1974).

5.4. Effect of heat stress on Biochemical parameters

Biochemical parameters are found variation related to changes in ambient temperature, and temperature-humidity index, although within the physiological range for cows which is found by Grünwaldt *et al*., (2006). Different biochemical parameters were studied in May and variations in parameters were found in stressed cows at different lactation stages which has corresponded with the statement by Abdelatif *et al.*, (2012). Yousuf *et al*., (2014) showed that the values of carbohydrate and protein levels of different lactating stressed animals are changed due to heat stress. The highest values of Glucose, Total Protein, and Albumin were (25.05± 6.39 mg/dl); (85.92±19.52 mg/dl); (40.14 ±5.25g/L), respectively, in 3^{rd}, 5^{th} and 4^{th} lactating stressed cows in May (Ndlovu *et al.,* 2007). The values of Glucose, Total Protein, and Albumin found in stressed lactating cows were insignificant (P>0.05).

The values of minerals in heat-stressed cows were the study findings. The highest values of Na, P, Ca, and Cl of stressed animals were 135.18±10.35 mmol/L, 6.88±2.83 mmol/L, 8.97±0.99 mg/dl, and 121.59±8.43 mmol/L, respectively of 3^{rd}, 5^{th}, 4^{th} and 4th lactating cows in May (Berian *et al*., 2019). In addition, no significant (P>0.05) variation among values of Na, P, Ca, and Cl was observed in milking cows at different lactation periods (Abdelatif and Alameen, 2012). The study shows the values of enzymes in heat-stressed lactating cows monitored in May. The highest values of blood ALT and AST of stressed animals were 39 ±3.43 U/L and 89.125 ±18.84 U/L, respectively of 5^{th} lactating cows in May (Prathap *et al*.,2017). This study also showed a significant ($P<0.05$) variation in blood AST levels among cows at different lactation periods (Kadzere et al., 2002).

The biochemical parameters of all the samples were observed in heat-stressed cows during May. The values of Glucose, Total Protein, Albumin, Na, Ca, Cl, ALT and AST was in normal valuer range whereas P value was higher which has stated with Feldman *et al*., (2002).

Chapter VI: Conclusion

The present study revealed highest ambient, cow shed and rectal temperature of cows in the month of May 2022. The productive performance and haemato-biochemical parameters of intensively reared dairy animals are affected by heat. The ambient temperature directly affects the body physiology of cows—reflected by the increased rectal temperature and decreased milk production. Milk parameters like fat, SNF were significantly changed and total solids insignificantly changed due to heat stress. It also affects the older cows (5^{th} lactation) than the younger animals (3^{rd} lactation) which is reflected through the changes of hematological parameters (especially WBC) and biochemical parameters (especially AST). Therefore, there could be possible effect of the ambient temperature on the heat stress of dairy cows and optimum environmental temperature is necessary for the better production and body physiology of intensively reared dairy cows.

Chapter VII: Recommendations

Intensive intervention study needs to carried out to know the haemato-biochemical level in the month of March and April. There should need to control monitoring group of study population. It should be better if consider feeding, medication, management and budgetary system.

Chapter VIII: References

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Appendices

Table: 12 Average Milk Production

Cow No	Shed No	Lactation	Average Milk Production March	Average Milk Production April	Average Milk Production May
50309	2	3rd	9.913333333	8.5	9.323333333
50753	2	3rd	9.263333333	8.213333333	8.54
50593	1	3rd	9.756666667	8.306666667	8.906666667
51944	1	3rd	11.13448276	10.47	11.19
50207	4	3rd	11.02666667	11.13333333	9.126666667
50842	1	3rd	11.53666667	9.98	10.18
50554	4	3rd	9.646666667	8.693333333	3.456666667
50702	3	3rd	9.843333333	9.686666667	1.64
50045	2	3rd	12.07333333	10.16	10.91666667
50483	1	3rd	11.38333333	8.136666667	9.656666667
50391	3	3rd	10.84666667	11	12.41666667
50303	1	3rd	9.823333333	8.943333333	9.283333333
50115	3	3rd	16.16666667	14.86666667	16.41333333
50885	2	3rd	17.00333333	14.92	15.19666667
50898	5	3rd	18.06	15.46666667	16.45
50424	5	3rd	15.23333333	12.76666667	12.56
50471	1	3rd	19.51	18.75666667	18.38333333
50864	3	3rd	20.17666667	16.97	17.43
50782	3	3rd	14.1	12.76666667	12.52
50865	4	3rd	14.75	13.61666667	12.56
50952	5	3rd	15.69333333	14.48333333	12.55
50787	3	3rd	12.51666667	11.84666667	10.59
50396	3	3rd	10.49333333	8.386666667	9.396666667

Table: 13 Comparison of shed, environmental (ambient) and rectal temperature:

Month	Ambient Temperature	P value	Rectal Temperature	P value	Lactation			P value
					3rd	4th	5th	
March	99.75± 0.570	0.1121	101.636 ± 0.460	0.2966	101.671 ± .446	101.587 ± .495	101.651 ±.450	0.7648
April	99.99 ± 0.605	0.0036	101.712 ± 0.344	0.2733	101.898 ±.304	101.876 ± .341	101.989 ±.382	0.4080
May	100.21± 0.408	0.0001	101.821 ± 0.364	0.2636	101.747 ± .384	101.911 ±.381	101.805 ±.316	0.2107

Table: 14 Comparison of Average Milk Production Between Month:

Comparison of Month	Average Milk Production	P value
March and April	11.600 ± 3.324	0.0000
March and May	10.704 ± 3.682	0.0329
April and May	10.704 ± 3.682	0.0000

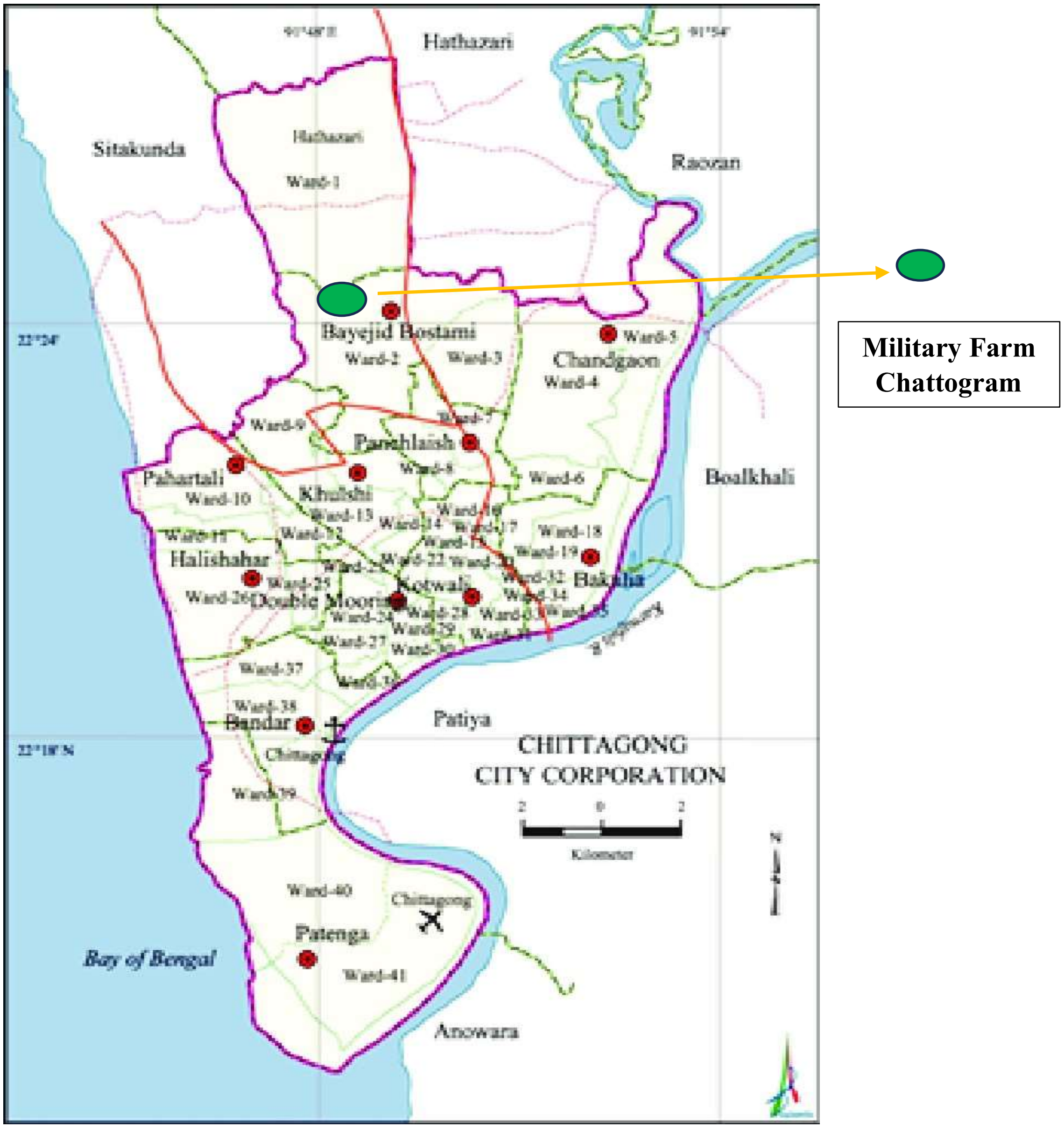

Fig.3: Chattogram District

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: Sheike Rezaul Karim

MS Fellow (Physiology)

Session: January- June 2022

SI 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 Milking Cows/ Parity /Dry Cows):

Type	Amount (kg)/animal
Concentrate	
Roughage	

1. ID:
2. Age:
3. Genotype (Cross):
4. Body weight:

5. Age:

6. Stage of Lactation:

7. Last date of Insemination:

8. Date of Calving:

9. Gestation period:

10. Milk Production:

11. Blood Biochemistry:

12. Parity:

13. Milk Parameter:

14. Blood Hematology:

15. Remarks:

<u>Biography</u>

Lt Col Dr. Sheike Rezaul karim passed the SSC examination in 1993 and then HSC examination in 1995. He obtained Doctor of Veterinary Medicine in 2003 from Chattogram Veterinary and Animal Sciences University. After completing his DVM degree he joined in Bangladesh Army on 20th July 2004. He passed out from Bangladesh Military Academy with BMTC-29.

He was commissioned in the prestigious and traditional RV&F Corps. After joining, he has served in all possible appointments as a junior officer. He served as Farm officer, Veterinary Officer in Military Farm Lalmonirhat, Military Farm Chattogram and Military Farm Savar. He has also served in Army Head Quarters, Supply and Transport Directorate as staff officer and as Officer in Charge in Armed Forces Institute of Pathology. He has participated in the United Nations Peacekeeping Mission in Democratic Republic of Congo and visited Uganda, Kenya, India, Australia, Ireland and Turkey. Recent past he has served as second in command of military farm Jashore. Presently, he is serving as Commanding Officer of Adhoc Military Farm Cox's bazar, Ramu Cantonment.