Table of contents

Contents **Page No.**

List of tables…… II

Abstract ……..…….…….... III

Chapter 1: Introduction …….…..... (01-02)

Chapter 2: Materials and Methods

2.1 Study area and duration: ...…….…… 03

2.2 Study population: …….…….……..... 03

2.3 Sample collection and processing: …….…… 03

2.3 Statistical analysis: …….…….……... 04

Chapter 3: Results …….…….…..... (05-08)

Chapter 4: Discussion ……....…… (09-10)

Conclusion …….…… 11

Limitations …… 12

References ……..……. (13-16)

Acknowledgement…….…… 17

Biography ……....…….……....….…..... 18

List of Tables

Sl. No.	Title	Page No.
01	Hematological indices of crossbred pregnant and non-pregnant cows	**06**
02	Bio-chemical parameters of crossbred pregnant and non-pregnant cows	**07**
03	Hormonal indices of crossbred pregnant and non-pregnant cows	**08**

ABSTRACT

Haematological, biochemical and hormonal changes occur throughout the pregnancy to nurture the developing fetus and prepare the mother for parturition. The current study aimed to evaluate and compare several hematological, biochemical and hormonal parameters of late pregnant (N=10) and non-pregnant (N=10) dairy cattle at four dairy farms in Chattogram district. The selected animals were expected to have parturition within 0-15 days of blood collection. Blood samples were collected from tail vein and serum was separated for the analysis of biochemical metabolites and hormones by commercially available kits. Hematological indices showed that Hb, PCV%, MCH, PLT count differences between pregnant and non-pregnant cows were statistically significant ($P<0.05$). Others hematological parameters like TEC, MCV, MCHC, TLC, lymphocyte, monocyte and eosinophil counts were statistically non-significant in two groups. Among biochemical parameters only glucose was found to be higher in pregnant cows under this study. Furthermore, estrogen altitude in pregnant cows was found significantly higher ($P<0.05$) than the non-pregnant cows but progesterone level was non-significantly lower ($P>0.05$) in late pregnant cows. In conclusion, these results suggest that during late pregnancy hematological, blood metabolites and hormonal profile changes for the wellbeing of mother and fetus as well.

Keywords: Hematological; hormonal; blood metabolites; pregnant; non pregnant.

Chapter 1

INTRODUCTION

A dairy cow has several stages in its's whole life cycle e.g., calf, estrus, heifer, pregnancy, parturition, lactation, dry stage etc. Pregnancy is one of the most remarkable physiological events in all animal species that causes dramatic changes in hematological, hormonal and biochemical variables. Moreover, among the reproduction stages of a dairy cow, transition period is critical due to sudden endocrine and metabolic changes that cover parturition and the initiation of lactation (Gbolabo *et al.,* 2015). This period is also an important stage for dairy cows since the physiology of the dairy cow changes due to the rapid growth of the fetus (Bell, 2000). Sudden physiological alteration occurs in this stage, especially in the late gestation period. Any kind of imbalance may result in abortion as well as other kinds of economic losses. In bovine medicine, in combination with clinical examination and other diagnostic procedures, serum biochemistry is known as a highly informative diagnostic tool (Rahman *et al.,* 2006). Moreover, blood profile metabolites have been used widely not only for the identification of problems associated with the reproductive cycle but also for the evaluation of health status (Babak *et al.,* 2018). So far, serum bio-chemical and hematological indices constitute important panels in the diagnosis, prognosis and treatment of livestock diseases (Roland *et al.,* 2018). So, investigation of parameters influencing these blood and serum bio-chemical indices are important. Recently, hematology and biochemistry have become integral diagnostic tools in bovine medicine (Hagawane *et al.,* 2009). Practitioners tend to assess the biochemical parameters along with other supportive tools to solve any issues regarding the production and reproduction cycles. With the ever-increasing interest in commercial dairy farming, emphasis on the importance of physiological equilibrium maintenance in dairy cows is also increasing day by day (Otto *et el,* 2008). Physiological permanence is maintained by body fluid mainly by blood (Geneser, 1986), but some unusual conditions may alter this stability sometimes. When anamnesis and physical examination fail to reach the diagnosis, practitioners examine blood profile, serum biochemistry and even hormonal assay, hoping these tests will identify the problem (Navarre, 2007). Sometimes, differences seen in the blood levels of various parameters among pregnant and non-pregnant cattle due to genetic, climatic, nutritional and environmental conditions (Otto *et al.,* 1992). Expanded blood volume requires by the uterus during pregnancy period (Surabhi *et al.,* 2012). At this time, the

concentration of several blood constituents alter significantly, white blood cells and other biochemical indices are found higher in cattle (Abuelo *et al*., 2015). Genetic makeup, nutritional state, reproductive state (pregnancy, estrus, lactation), environmental factors etc. are known to affect hemato-biochemical parameters (Balikci *et al.,* 2007). Hence, the biochemical values during pregnancy should be known for the diagnosis of various pathological disorders, that may adversely affect the reproductive performance of cows, resulting in great economic losses to farmers (Pyne *et al.,* 1981). Pregnancy and maternal endocrine system interact during the gestation period for pregnancy maintenance along with fetal growth and development till parturition. The uterus of the cow undergoes morphological and functional changes during pregnancy (Bezerra *et al.,* 2008). Moreover, hormonal alterations that occur in the uterus regulate the immune cells and immunity of pregnant cows to some extent (Rahman *et al.,* 2006). Therefore, it is important to understand the hormonal interaction happens in the uterus of a cow during any stage of pregnancy. Because any hormonal imbalance can terminate pregnancy abruptly (Henricks *et al.,* 1972). However, the hormonal equilibrium is achieved by a variety of regulatory agents (protein and steroid hormones etc.) and also by the response of the maternal system to let normal fetal development. Huge changes in estrogens, progesterone, prolactin, glucocorticoids and growth hormone occurs during different stages of pregnancy (William *et al.,* 1980).

Therefore, the present study was performed

· To determine the hormonal, hematological and biochemical profile in pregnant (later stage) and non-pregnant cattle

· To compare the biochemical blood parameters of pregnant (later stage) and non-pregnant cattle.

Chapter 2

MATERIALS AND METHODS

2.1 Study area and duration:

The study was conducted for a period of 2 months (October 2019 to December 2019) in four selected dairy farms in Shikalbaha and Chattogram city, Bangladesh. The farms were selected based on record-keeping history, standard housing, standard ration feeding, immunization against infectious disease and acceptance of farm to contribute to research work.

2.2 Study population:

The study population was 20 crossbred (HF × Local) healthy cows, from which 10 pregnant (later stage) and 10 non-pregnant cows were selected. All animals involved in this study were clinically healthy to ensure this clinical history was reviewed with the farm manager through the examination of physical condition.

2.3 Sample collection and processing:

Blood samples were taken from tail vein by puncturing with proper aseptic measures. About 10 ml of blood was collected and kept in the vacutainer (with and without anticoagulant). The samples were transported to the Department of Physiology, Biochemistry and Pharmacology at Chattogram Veterinary And Animal Sciences University (CVASU), Bangladesh for analysis. Hematological indices viz. Total Erythrocyte Count(TEC), Hemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Total Leukocyte Count (TLC), Platelet (PLT), Lymphocyte (Lym), Monocyte (Mon), Eosinophil (Eos) were determined by the Nihon Kohdens hematology analyzer Celltac Alpha VET MEK-6550.

After coagulation, coagulated blood was centrifuged at 3000 rpm for 15 minutes. The Serum was transferred to the eppendorf tube by using a micropipette. The obtained serum samples were stored in -20ºC for biochemical test. Glucose, Total Protein (TP), Triglyceride (TG), Cholesterol, High Density Lipoprotein(HDL) of blood serum were determined by the Humalyzer 3000, Germany®.

Hormonal indices viz. Testosterone, Progesterone, Estrogen, Luteinizing Hormone (LH) value were obtained by supplied manufacturer instructions. Testosterone (BIOS Microwell diagnostic system), estrogen (Nova Tec Immunodiagnostica GmbH, Germany), progesterone (Nova Tec Immunodiagnostica GmbH, Germany) and LH (Atlas Medical, UK)

2.4 Statistical analysis:

Obtained laboratory data was stored in Microsoft Excel-2010. Descriptive statistical analysis was done to measure the mean, variance, standard deviation, and p-value of different parameters. PAIRED T-TEST was done to assess the hemato-biochemical and hormonal parameters in crossbred pregnant and non-pregnant cows. In all cases, the level of significance was determined at $P<0.05$.

Chapter 3

RESULTS

Hematological profile in pregnant and non-pregnant cows are presented in Table 1. Hemoglobin, PCV and MCH were significantly higher in late pregnancy compare to non-pregnant cows, (9.14±.0.73 gm/dl) vs (8.08±1.13 gm/dl), $P<0.05$, (25.72±2.11%) vs (22.85±3.20%), $P<0.05$ and (15.78±0.97%) vs (14.95±0.74%), $P<0.05$. (Table 1).

On the other hand, mean level of other parameters altered insignificantly in two study groups. TEC, MCV, MCHC, lymphocyte % and Monocyte % in pregnant cows were (5.71±0.68x10^6/µl), (44.31±2.65cumm), (35.63±0.56%), (42.97±11.86%) and (0.73±1.26%) respectively (Table 1). Which was insignificantly higher than the non-pregnant cows (5.44±0.91x10^6/µl), (42.24±2.79 cumm), (35.36±.0.84%), (39.39±9.99%) and (0.07±0.11%) respectively (Table 1). Conversely, TLC and eosinophil % was insignificantly increased in non-pregnant cow than the pregnant one, (9.05±2.79x10^3/µl) vs (8.26±2.26x10^3/µl), (6.25±3.37%) vs (5.54±4.25%), (Table 1). But, the platelet count is significantly higher in non-pregnant cows than the pregnant cows, (281±130.45) vs (181.4±78.43), $P<0.05$, (Table 1).

Table 1 Hematological indices of crossbred pregnant and non-pregnant cows (N=10)

Parameter	Pregnant cow Mean±SD	Non-pregnant cow Mean±SD	P-value
TEC(x10^6/µl)	5.71±0.68	5.44±0.91	0.23
Hb(gm%)	9.14±0.73	8.08±1.13	0.01
PCV(%)	25.72±2.11	22.85±3.20	0.02
MCV(cumm)	44.31±2.65	42.24±2.79	0.05
MCH(%)	15.78±0.97	14.95±0.74	0.02
MCHC(%)	35.63±0.56	35.36±0.84	0.20
TLC(x10^3/µl)	8.26±2.26	9.05±2.79	0.25
PLT count	181.4±78.43	281±130.45	0.03
LYM(%)	42.97±11.86	39.39±9.99	0.24
MON(%)	0.73±1.26	0.07±0.11	0.07
EOS(%)	5.54±4.25	6.25±3.37	0.35

**Significant = P<0.05; Nonsignificant = P>0.05 (PAIRED T- TEST)

**TEC =Total Erythrocyte Count, Hb = Hemoglobin, PCV = Packed Cell Volume, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration, TLC = Total Leukocyte count, PLT = Platelet, Lym = Lymphocyte, Mon = Monocyte, Eos = Eosinophil

Serum biochemical changes in the study groups (pregnant and non-pregnant) are depicted in Table 2. The level of glucose was found to be changed at later stage of pregnancy. The glucose level in pregnant and non-pregnant cows was **(**70.89±10.72 mg/dl) and (63.94±10.54 mg/dl) respectively (Table 2).

During later stage of pregnancy serum total protein, triglycerides, cholesterol and high density lipoprotein were decreased in pregnant animals (47.3±3.14 mg/dl, 56.51±11.15 mg/dl, 117.01±31.01 mg/dl and 97.73±22.15 mg/dl, respectively) than the non-pregnant animals (55.54±2.73 mg/dl, 59.83±52.56 mg/dl, 171.05±100.94 mg/dl and 121.4±81.62 mg/dl respectively) (Table 2).

Table 2 Biochemical parameter of crossbred pregnant and non-pregnant cows (N=10)

Parameter	Pregnant cow Mean±SD	Non-pregnant cow Mean±SD	P-value
Glucose (mg/dl)	70.89±10.72	63.94±10.54	0.08
TP (mg/dl)	47.3±3.14	55.54±2.73	3.38
TG (mg/dl)	56.51±11.15	59.83±52.56	0.42
Cholesterol (mg/dl)	117.01±31.01	171.05±100.94	0.07
HDL (mg/dl)	97.73±22.15	121.4±81.62	0.20

**Significant = P<0.05; Nonsignificant = P>0.05 (PAIRED T- TEST)

**TP = Total Protein, TG = Triglyceride, HDL = High Density Lipoprotein

Hormonal indices of pregnant and non-pregnant cows were given in Table 3. Testosterone, Estrogen and LH concentration were found to be higher during late pregnancy (0.32±0.25), (80.97±35.49 and (1.38±1.49) respectively, but only estrogen level was significantly higher in pregnant cows (80.97±35.49), P<0.05 than the non-pregnant cows (Table 3). On the other hand, Progesterone level was found higher in non-pregnant cows (21.35±17.34) than the pregnant cows (20.08±9.98) at an insignificant level (Table 3).

Table 3 Hormonal indices of crossbred pregnant and non-pregnant cows (N=10)

Parameter	Pregnant cow Mean±SD	Non-pregnant cow Mean±SD	P-value
Testosterone	0.32±0.25	0.29±0.39	0.41
Progesterone	20.08±9.98	21.35±17.34	0.42
Estrogen	80.97±35.49	50.39±25.75	0.02
LH	1.38±1.49	0.55±0.36	0.06

**Significant = P<0.05; Nonsignificant = P>0.05 (PAIRED T- TEST)

**LH = Luteinizing Hormone

Chapter 4

DISCUSSION

Notable changes in several biochemical and hematological values during late pregnancy were observed in this study. Our study provides some significant information regarding physiological changes that occur during late pregnancy.

In our study, we selected the pregnant cows which were 0 to 15 days before parturition. Our results show significantly higher Hg (%), PCV (%) and MCH (%) in pregnant cows than the non-pregnant cows. And PLT count in pregnant cows was found significantly lower than the non-pregnant cows (Table 1). A previous study on pregnant cows reported, increased level of hemoglobin at late gestation that may be related to increased oxygen flow due to fetal growth and cows' requirements (Abud *et al.,* 2016). Moreover, PCV and hemoglobin concentration are proportional to each other. One study in pregnant cows found PCV and Hb % were significantly higher in postpartum cows (Nazifi *et al*., 2008). Conversely, a result recorded by (Flores *et al.,* 2013) found a nonsignificant difference in hematocrit values between dry(non-lactating) pregnant cows and lactating cows. In our study, a significantly higher level of MCH was observed in pregnant cows than the non-pregnant cows. MCH value in pregnant dry (non-lactating) cows was found higher 65.07 ± 2.27 (fl) than the non-pregnant dry (non-lactating) cows 57.93 ± 2.15 (fl) Sattar *et. al.*, (2009). Kumar *et al.,* (2000), reported the highest MCV, MCH and lowest MCHC in non-pregnant dry (non-lactating) cows compared to non-pregnant cows. In our study, we found a statistically significant difference in PLT count between pregnant and non-pregnant groups. Non-pregnant cows show a higher level of PLT count than the pregnant cows (Table 1). Johnson *et al.,* (1990), reported statistically significant differences in platelet and white blood cell count between the two groups. Multiple physiological changes during pregnancy may result in lower platelet counts. Dilution of platelets due to increased plasma volume that occurs during pregnancy to support fetal growth may be the probable cause of lower PLT count in all mammals during pregnancy (Abdul *et al.,* 2012).

We know, glucose is a sugar that circulates in the blood serving as the body's main source of energy. It is regarded as one of the indicators of energy status in a cow. Table 2 shows that, glucose level was insignificantly increased during pregnancy period. A similar pattern has been reported in the study of Peterson *et al.,* (1981), glucose level was higher in pregnant cows (69.8±3.7 mg/dl) than the non-pregnant cows (65.2±1.7 mg/dl). The higher

blood glucose level during pregnancy may be due to high energy diet feeding and also provision of the extra amount of feed than the requirement for pregnancy maintenance (Samun *et al*., 2015). The total protein level in pregnant cows was lower than the non-pregnant cows. Which is contradictory to Giuseppe *et al.,* (2012). Who reported that, total serum protein in pregnant cows was slightly higher than the non-pregnant cows. Cholesterol and HDL concentration were found lower in pregnant cows that agree with the findings of Zafar *et al.*, (2020), where serum cholesterol level was lower in the pregnant cows 14.18±5.75 (mg/dl) than the non-pregnant cows 37.43±7.87 (mg/dl). Advancement of the lactation stage results in rise of cholesterol level to meet the requirements of lactation. Due to the physiological adjustment of pregnancy estrogen along with thyroxin play an important role in reducing cholesterol level (Rowland *et al.*,1998).

Estrogen and progesterone are the chief pregnancy hormones (Henricks *et al.,* 1972). Table 3 exhibits, slightly lower concentration of progesterone hormone in pregnant cows than the non-pregnant cows. Advancement of gestation causes the preparation of the body for parturition that results in fall of progesterone concentration and rise of estrogen concentration in the blood slowly (Rahman *et al*., 2006). A statistically significant difference in the estrogen hormone level was found in our study because our selected population was in the last 0 to 15 days before parturition. A similar study on deer by Hoffmann *et al.,* (1978) reported, statistically significant difference in estrogen level in pregnant and non-pregnant does. Estrogen level during pregnancy reaches its peak during late gestation ((Rahman *et al.,* 2006). The increase in estrogen concentration during pregnancy enables the uterus and placenta to improve vascularization, transfer nutrients and plays a crucial role to develop and mature fetus (Levitz *et al.,* 1978). Enzyme systems of placenta make estradiol from three different neutral steroids which is also associated with rise in estrogen level during pregnancy (Ryan, 1959). Insignificantly higher level of testosterone and LH hormone observed in pregnant cows under study. A similar pattern has been reported in the study of O'Leary *et al.,* (1991), maternal serum testosterone concentrations increase by 70% during pregnancy. Mongkonpunya *et al.,* (1975) reported that, peripheral plasma concentration of testosterone was slightly higher in cows with male rather than female fetuses. A study by Mondal *et al.,* (2019) reported, mean LH concentration in anestrous or pregnant group is lower than the non-pregnant and cyclic group. Which is contradictory with the findings of our study.

CONCLUSION

The current study found a significant level of changes in hematological (Hb, PCV%, MCH %, PLT count), biochemical and hormonal (estrogen) profile among pregnant and non-pregnant crossbred cows. The analyzed parameters indicate that there was an alteration of serum bio-chemical levels in the study population at different physiological stages. For a comprehensive evaluation of the values of different parameters at different stages, further studies should be carried on.

LIMITATIONS

Being an undergraduate student I have faced some problems during this study. The sample size was only 20. Due to a very small sample size it was quite difficult to interpret the exact hematological, biochemical and hormonal parameters in different physiological condition among pregnant and non-pregnant cows precisely. Moreover, due to time limitation I was not able to compare all three stages of pregnancy with non-pregnant one. For better evaluation of the tested parameters, a larger sample size is recommended.

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ACKNOWLEDGEMENT

The author is ever grateful and indebted to the Almighty Allah without whose grace it would have never been possible to pursue this study in this field of science and to complete this clinical report writing for the Degree of Doctor of Veterinary Medicine (DVM).

The author would like to express her deep sense of gratitude and heartful appreciation to Professor **Dr. Goutam Buddha Das**, Vice- Chancellor of Chattogram Veterinary and Animal Sciences University.

The author would like to express her deep sense of gratitude and heartfelt appreciation to Professor **Dr. Abdul Ahad**, Dean, FVM, Chattogram Veterinary and Animal Sciences University.

It is deemed as a proud privilege and extra-terrestrial pleasure to express authors ever indebtedness, deepest sense of gratitude, sincere appreciation and profound regards to authors reverend and beloved teacher and Supervisor **DR. Jabin Sultana**, Assistant Professor, Department of Physiology, Biochemistry & Pharmacology, Chattogram Veterinary and Animal Sciences University for her scholastic guidance, uncompromising principles, sympathetic supervision, valuable advice, constant inspiration, affectionate feeling, radical investigation and constructive criticism in all phases of this study and preparing the manuscript also.

The author highly expresses her gratitude and thanks to **Prof. Dr. A.K.M. Saifuddin**, Director, External Affairs, Chattogram Veterinary and Animal Sciences University, for his constant inspiration, cordial co-operation, valuable suggestion for completion of the report work.

The author is ever indebted to her father, mother, brothers, sisters, uncles, grandmother, grandfather and other relatives for their sacrifices, blessing and encouragement to get her in this position.

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September, 2020

BIOGRAPHY

I am Farnaz Kader Nova, daughter of Md. Abdul Kader and Mrs. Hamida Khanam. I passed my Secondary School Certificate (SSC) examination from Chattogram Govt. Girls' High School, Chattogram with G.P.A -5.00 and Higher Secondary Certificate (HSC) examination from Bangladesh Women's Association School and College with G.P.A-5.00. Now, I am an intern veterinarian under the Faculty of Veterinary Medicine at Chattogram Veterinary and Animal Sciences University. I have an immense interest to work in the field of Genetics and Breeding.