Circulating Concentration of Certain Blood Constituents in Crossbred Heifers

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

(FACULTY OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY)

PUSA (SAMASTIPUR), BIHAR

By

Jitendra Kumar Deepak

In partial fulfilment of tbe requirements

FOR THE DEGREE OF

Master of Veterinary Science

(VETERINARY PHYSIOLOGY)

POST GRADUATE DEPARTMENT OF VETERINARY PHYSIOLOGY

BIHAR VETERINARY COLLEGE

PATNA - 800 014

2003

Registeration No.- M/VPY-22/1999-2000

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2003

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DEDICATED

to my

GRAND PARENTS

Dr. C. Singh
M.V.Sc., Ph.D.
Associate Professor & Head

Department of Veterinary Physiology
Bihar Veterinary College, Patna-800014
(Rajendra Agricultural University, Pusa, Bihar)

CERTIFICATE - I

This is to certify that the thesis entitled "**CIRCULATING CONCENTRATION OF CERTAIN BLOOD CONSTITUENTS IN CROSSBRED HEIFERS**" submitted in partial fulfillment of the requirement for the award of **Master of Veterinary Science** in **Veterinary Physiology**, Rajendra Agricultural University, Bihar is the record of bonafide research work carried out by **DR. JITENDRA KUMAR DEEPAK** under my supervision and guidance. No part of the thesis has so far been submitted for any other degree or diploma.

It is further certified that the assistance and help received during the course of this investigation and the sources of literature have been fully acknowledged.

25/08/03

[Dr. C. Singh]
Major Advisor

CERTIFICATE - II

We, the undersigned, members of the Advisory Committee of **DR. JITENDRA KUMAR DEEPAK**, a candidate for the degree of **Master of Veterinary Science with major in Veterinary Physiology** have gone through the manuscript of the thesis and agree that the thesis entitled "**CIRCULATING CONCENTRATION OF CERTAIN BLOOD CONSTITUENTS IN CROSSBRED HEIFERS**" may be submitted by **DR. JITENDRA DEEPAK** in partial fulfillment of the requirements for the degree.

25/08/03

[Dr. C. Singh]
Chairman
Advisory Committee

Members of the Advisory Committee :

1. **Dr. C. Singh**
   Assoc. Prof. & Head,
   Veterinary Physiology

25/08/03

2. **Dr. J. N. Singh**
   Assoc. Prof. & Head
   Department of LPT

25/08/03

3. **Dr. S. B. Verma**
   Assoc. Prof.
   Department of ABG

25/8/03

Endorsed :-

25.08.03

Dr. C. Singh
Assoc. Prof. & Head,
Deptt. of Veterinary Physiology,
Bihar Veterinary College, Patna-14

Dr. H. Akhtar
(Dean, P.G. Nominee)
Assoc. Prof. & Head
Deptt. of Veterinary Obstetrics & Gynaecology

CERTIFICATE - III

This is to certify that the thesis entitled "**CIRCULATING CONCENTRATION OF CERTAIN BLOOD CONSTITUENTS IN CROSSBRED HEIFERS**" submitted in partial fulfillment of the requirements for the degree of Post-Graduate studies, Rajendra Agricultural University, Bihar, Pusa, was examined and approved on _____ 2003.

11/02/04

[Dr. C. Singh]
Chairman
Advisory Committee

_____
External Examiner

Members of the Advisory Committee :

1. **Dr. C. Singh** 11/02/04
2. **Dr. J. N. Singh** 11/02/04
3. **Dr. S. B. Verma** 11/02/04

11.02.04

Dr. H. Akhtar
(Dean, P.G. Nominee)

_____
Dean, Post-Graduate Studies

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Place : Patna

Date : **(Jitendra Kumar Deepak)**

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Chapter - 1

Introduction

INTRODUCTION

Animal husbandry has been practiced in India from the time immemorial. The selection of animals for higher productivity has been a specialised endeavour since the time of development of consciousness for animal farming. The efforts and art of rearing of animals under the diversed agroeconomical conditions of the country have led to the development of highly diversed livestock genetic resources over the centuries. This is evident from the availability of most of the species of farm animals, and an usually large number of genetic variants in each species. Presently India has 26 well defined cattle breeds among which 4 are milch breeds, 8 dual purpose and rest 14 are draught purpose. The greatest constraints with our indigenous breeds of cattle is they have poor production and reproduction potential inspite of providing adequate and ideal management practices. The per animal milk production from our indigenous cows is lower than the exotic breeds of cows maintained in temperate climate.

Besides, the lower milk producing potential of our native breeds the age of puberty and calving intervals are also higher than the European breed of cattle.

However, Indian breeds show a markedly superiority in utilising poor quality feed, withstanding heat and resistance to

tropical diseases. Tropical breeds have thrived well through diverse situation over the generations inspite of a number of constraints and limitations. Thus they are adapted to this climate.

The demand of present era is to develop ways and means for increasing their productivity. The low productivity is due to gradual breed deterioration from general neglect over centuries, the consequent rise in the population of nondescript dairy animals, lack of adaptability and sustainability of crossbred cattle in the varied agroclimatic condition of country, lack of breeding policy for cross breeding programmes and poor knowledge of dairy husbandry men of rural area in respect of scientific and modern management of the newly evolved crossbred animals. Except in few organised farms which maintain small herds of pure breed there is almost unrestricted interbreeding among different breeds. Some of the indigenous breed have already been diluted or eroded, while in others, unique genetic characteristics are declining. From the estimated number of breedable female available the endangered milk producing indigenous breeds are Red Sindhi, Sahiwal and Tharparkar. These breeds have extensively used for upgrading native nondescript and other low yielding animals. Hariana has made the largest impact on cattle in the Indo Gangatic Plains, extending from its home in Haryana and Punjab to west Bengal. It has been extensively used for upgrading local breeds to improve their draught and milch qualities.

In order to bring about rapid genetic improvement crossbreeding of indigenous cattle with high milk producing and early maturing exotic temperate breeds was recommended and at this era of scientific development it is essential to study the intrinsic mechanism controlling the process of growth and reproduction in crossbreed animals. Metabolic profile testing has been used to monitor individual and dairy herd health. The knowledge of blood chemistry of dairy animals is of great clinical importance in assessment of their health, nutritional status, diagnosis and progresses of metabolic diseases.

Some earlier workers (Arosh *et al.*, 1998; Sharma *et al.*, 1998; Kabir *et al.*, 2001, Manowar and Singh, 2002; Rathee *et al.*, 2002) have established the relation of different hematological and biochemical profiles with growth and estrous cycle of animals, even though the works on physiological adaptation of crossbred animals in tropical climate are very limited. The works on different blood constituents of animal with relation to their reproductive cycle are very few. Earlier workers in our laboratory (Prabha et al., 1999; Manowar and Singh, 2002) have indicated that the hemograms and certain blood constituents of crossbred calves and heifers in tropical climate were not similar to the constituents reported in pure breed animals of temperate climate. This indicates non adaptability of these crossbred animals in tropical climate.

Very few work so far has been done on the evaluation of intrinsic physiological mechanism and profiles of physiological constituents prevailing in the circulation of newly evolved high yielding crossbred animals. The production and reproduction trait of dairy animal depends on the internal physiological rhythm of dependent organ of respective trait. The normal physiological rhythm of any organ can be evaluated by means either of its potential for substrate utilisation, organ composition or release of its products in circulation and maintenance of these products for different productive and reproductive performance. Thus the circulating level of different hematological and Biochemical constituents at different ages and stages of reproduction may be a guiding factor for the climatic adaptation of these crossbred cattle in tropical climate of Bihar. Hence there is need of thorough investigation of the level of biochemical constituents of blood, which may reflect the changes in production due to some difference in circulating constituents. Thus, the present study has been undertaken to study the circulating blood constituents in crossbred heifers maintained in cattle farm, Pusa. The main area of study for the present investigation was as follows.

1. Estimation of the haemogrammes viz, BCT, Hb, TEC, ESR, TLC, DLC, PCV, MCH, MCV and MCHC to study its relation with age and cyclicity.

2. To estimate the concentration of sodium and potassium in cycling and noncycling crossbred animals.

3. To estimate the biochemical constituents like total serum protein, creatinine, and uric acid in cycling and non cycling crossbred animals.

4. To estimate the SGOT, SGPT and alkaline phosphatase activities in these crossbred animals.

□□□□□

Chapter - 2

Review of Literature

REVIEW OF LITERATURE

2.1 Hemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).

Mishra and Biswal (1961) reported the TEC, Hb and PCV in Orissa cattle as 6.50 ± 1.06 million/mm^3, 12.5 ± 2.05 g% and 38.00 ± 7.87 % respectively. They observed fluctuation without trend in different values with age, sex and lactation.

Olbrich *et al.* (1971) reported the value of TEC, Hb, PCV, MCV, MCH and MCHC in Zebu cattle (Heat tolerant) as 9.90 mill/mm^3, 12.3 g%, 37.1%, 37.7 μ^3, 12.3 pg and 33.1% respectively. The same parameters in cold tolerant (Scotch highland) were 8.91 mill/mm^3, 13.7 g%, 41.5%, 47.3μ^3, 15.6 pg and 32.9% respectively.

Schalm et al. (1975) estimated the mean blood value in purebred Jersey female cattle and reported that in 3 ½ -4 ½ months. 7 ½ - 9 months, 11-12 months, 15-19 months, 20-30 months, 3-4 years, 4-6 years and 6-14 years the RBC value in million/mm^3 was 13.10, 10.65, 8.62, 9.15, 7.50, 8.70, 7.89 and 7.47 respectively ; Hb value (g%) were 11.07, 10.10, 9.60, 10.97, 10.70, 11.30, 11.20 and 11.10; PCV value (%) were 36.16, 30.40, 28.10, 34.30, 34.80, 40.00, 38.70 and 37.40 and MCV (μ^3) were 27.6, 28.5, 32.6, 38.0, 46.4, 46.0, 49.2 and 50.0 ; MCH (pg) were 8.45, 9.48, 11.13, 11.99, 14.30, 13.00,

14.20 and 14.86; and MCHC value (%) were 30.6, 33.2, 34.0, 31.5, 30.7, 28.2, 28.8 and 29.7 respectively.

Rao *et al.* (1981) reported the PCV value in Ongole cows in normal cycling, pregnant below 3 months, pregnant above 3 months, post parturient and repeater as 28.94 ± 0.93%, 28.37 ± 1.59%, 28.59 ± 0.72%, 26.83 ± 0.95% and 21.10 ± 4.04% respectively.

Shukla *et al.* (1982) studied the different hemograms in dry, pregnant and lactating Marwari ewes and reported the hemoglobin levels as 6.4 ±0.17 g%, 6.2 ± 0.39 g% and 5.6 ± 0.36 g% respectively. The TEC (million/mm^3) were 5.84 ± 0.36, 4.86 ± 0.74 and 6.22 ± 0.92. However, in both the groups the difference was non significant.

Naidu and Rao (1982) reported Hb in cycling and non cycling heifers (above 18 months) as 10.93 ± 1.52 g% and 8.60 ± 0.84 g% while in cycling and non cycling (90 days post partum) cows it was 10.25 ± 1.45 and 8.39 ± 0.92 g% respectively.

Borvonsin and Sirikhajornbhandhu (1983) reported the different hemograms in 10-19 months old heifers as Hb 11.69 ± 0.17 g%, PCV 31.19 ± 0.61% and TEC 7.12 ± 0.18 × $10^6/mm^3$.

Sharma *et al.* (1985) studied the hematological value of Murrah buffaloes of different ages and reported that in new born calves (0-3 months), young calves of 3-6 months, 6-12 months, growing calves of 12-24 months, Heifers (2-4 years) and adult

buffaloes (above 4 years) the TEC (million/mm^3) were 8.35 ± 0.31, 6.50 ± 0.70, 7.85 ± 0.58, 8.15 ± 0.34, 7.80 ± 0.38 and 7.20 ± 0.90 ; Hb (g%) were 14.98 ± 1.50, 11.98 ± 1.98, 12.01 ± 2.01; 11.55 ± 1.08, 12.10 ± 1.36 and 12.78 ± 0.85 ; PCV (%) were 45.10 ± 6.50, 36.80 ± 3.80, 37.50 ± 8.50, 36.15 ± 3.95, 39.80 ± 8.79 and 40.20 ± 6.80 ; MCV (μ^3) were 54.01 ± 3.91, 56.60 ± 4.36, 47.77 ± 2.01, 44.35 ± 2.35, 51.02 ± 3.82 and 55.83 ± 3.09 ; MCH (pg) were 17.94 ± 1.80, 18.43 ± 2.15, 15.30 ± 1.36, 14.17 ± 2.60, 15.51 ± 2.80 and 17.75 ± 1.08 ; and MCHC (%) were 33.15 ± 3.85, 32.55 ± 4.50, 32.02 ± 1.50, 31.95 ± 1.85, 30.40 ± 3.06 and 31.79 ± 1.76 respectively.

Deshpande *et al.* (1987) studied the different hemograms in different aged Red Khandhari cattle and reported that Hb, PCV, TEC, MCV, MCH and MCHC in non-pregnant non-lactating cows were 11.71 ± 0.26 g%, 34.86 ± 1.05 %, 3.97 ± 0.23 mill/mm^3, 93.52 ± 5.52 μ^3, 32.05 ± 2.04 pg and 33.47 ± 0.52 %. The same values in female calves were 11.18 ± 0.70 g%, 34.22 ± 1.67 %, 3.85 ± 0.43 mill/mm^3, 102.01 ± 16.60 μ^3, 35.43 ± 6.02 pg and 34.15 ± 1.34% while these levels in heifers were 12.41 ± 0.33 g%, 40.14 ± 0.74 %, 5.73 ± 0.34 mill/mm^3, 71.69 ± 4.72 μ^3, 21.97 01± 0.98 pg and 30.93 ± 0.84 % respectively.

Misra and Prusty (1989) studied the hematological level in 12-15 months Jersey heifers and reported that the Hb, PCV and TEC was 10.85 ± 0.38 g%, 31.20 ± 1.13% and 5.23 ± 0.39 mill/mm^3 respectively.

Gujar *et al.* (1990) reported that the Hb level in fertile estrus group (11.32 ± 0.21 g%) was significantly higher than non fertile (10.41 ± 0.20 g%) Kankarej heifers. The PCV in fertile group (38.35 ± 0.71%) was non significantly higher than non fertile (36.33 ± 0.98%) Kankarej heifers.

Kumar *et al.* (1990) studied the hemograms in female Murrah buffalo at birth, 1 month, 6 months, 12 months, 20-24 months and 25-30 months. The mean value of Hb level (g%) was 14.16 ± 0.59, 11.06 ± 0.65, 13.48 ± 0.28, 10.35 ± 0.78, 14.23 ± 0.51 and 13.29 ± 0.42 and PCV values (%) were 42.65 ± 3.74, 26.66 ± 5.25, 42.50 ± 1.98, 33.78 ± 2.83, 41.41 ± 0.44 and 39.08 ± 0.74; TEC (million / mm^3) were 7.01 ± 0.91, 4.11 ± 1.64, 7.74 ± 0.73, 5.56 ± 0.62, 7.46 ± 0.62 and 7.10 ± 0.72. MCV (μ^3) were 58.25 ± 6.54, 84.00 ± 5.58, 52.71 ± 1.61, 61.80±7.11, 67.11 ± 0.10 and 66.75 ± 5.25 MCH (pg) were 26.51± 5.69, 39.49 ± 11.15, 18.55 ± 1.86, 19.23 ± 2.12, 24.49 ± 0.04 and 24.02 ± 2.23 and MCHC (%) were 41.26 ± 3.99, 44.63 ± 3.97, 34.44 ± 2.32, 33.98 ± 2.00, 32.71 ± 0.02 and 33.46 ± 1.03 and respectively.

Ali *et al.* (1991) reported the Hb level in cyclic and anestrus rural crossbred heifers as 10.92 ± 0.34 g/dl and 7.92 ± 0.25 g/dl respectively.

Kumar *et al.* (1991) reported significant lower values of TEC, Hb and PCV in anoestrus and repeat breeder buffaloes. TEC (million/mm^3) were 6.87 ± 0.83, 5.13 ± 0.82 and 5.16 ± 0.78; Hb (g%)

were 12.02 ± 1.18, 9.03 ± 1.29 and 9.13 ± 1.22 and PCV (%) were 38.12 ± 5.64, 28.98 ± 4.13 and 31.03 ± 5.25 in normal cyclic, anoestrus and repeat breeder group respectively. MCH and MCHC did not differ in anoestrus and repeat breeder buffaloes from that of normal cyclic buffaloes. MCV value increased significantly in anoestrus and repeat breeder compared to normal cyclic group.

Khan *et al.* (1995) did not detected any change in Hb, TEC, PCV, MCV, MCH and MCHC among regular breeding, repeat breeding and anoestrus cows. The values were within normal physiological range. However, the regular breeding cows show a non-significant increase in Hb, MCH and MCHC as compared to repeat breeding and anoestrus cows.

Pradhan *et al.* (1995) reported the hemoglobin level in post partum anoestrus cows and normal cycling cows as 9.57 ± 0.25 g% and 11.74 ± 0.60 g% respectively.

Ramakrishna (1997) reported the hemoglobin concentration in anoestrus and cycling crossbred cows as 9.1 ± 0.80 g/dl and 10.43 ± 0.03 g/dl respectively.

Prabha *et al.* (1999) studied the hemograms in preparturient crossbred cows between 6-8 years of age, calves at birth, at one month, at 4-5 months and at 10-11 months and reported Hb (g%) as 9.16 ± 0.09, 10.63 ± 0.21, 10.20 ± 0.033, 12.26 ± 0.16 and 12.01 ± 0.20 ; PCV value (%) 32.36 ± 0.64, 23.36 ± 0.32, 19.73 ±

0.16, 31.33 ± 0.55 and 28.16 ± 1.04 ; TEC (million/mm^3) 4.87 ± 0.06, 5.66 ± 0.06, 5.35 ± 0.19, 6.33 ± 0.07 and 6.05 ± 0.10 ; MCV (μ^3) 66.35 ± 0.95, 41.18 ± 0.69, 37.01 ± 1.13, 49.38 ± 0.49 and 46.45 ± 1.03 ; MCH value (pg) 19.66 ± 0.08, 18.71 ± 0.38, 19.03 ± 0.42 and 19.81 ± 0.06 and MCHC value (%) 29.70 ± 0.33, 45.53 ± 1.29, 50.35 ± 0.43, 39.55 ± 0.31 and 42.78 ± 0.95 respectively.

Shrikhande and Sarode (1999) reported the Hb content in cows below 5 years, 5-8 years and above 8 years as 9.96 ± 0.32 g%, 9.81 ± 0.24 g% and 9.63 ± 0.31% respectively.

Khadjeh and Papahn (2002) observed highest (8.97 ± 1.746 × 10^6/mm^3)TEC in 0-6 months age of male buffaloes and the lowest (6.485 ± 1.642 × 10^6/mm^3) in 25-72 months of female buffaloes. Sex had no significant effects on other hematological parameters. Overall mean value of Hb, PCV, MCV, MCH and MCHC were 12.17 ±2.22 mg/dl, 34.86 ± 6.33% 47.78 ± 6.27 μ^3, 16.63 ± 1.68 pg and 35.29 ± 3.79 % respectively. Mean value of Hb and PCV decreased significantly with age while MCH and MCHC value increases with age non significantly.

2.2 Blood Clotting Time (BCT) and Erythrocyte Sedimentation Rate (ESR) :-

Ferguson (1937) while determining the ESR in cattle reported it as 2.4 mm per 7 hour.

Greatorex (1954) estimated the BCT in neonatal calves from birth to one year of age. The BCT at birth, 1 month, 4 month and 10 month were 5.6 minutes, 4.6 minutes, 5.1 minutes and 6.6 minutes respectively.

Mishra and Biswal (1961) found the coagulation time in Orissa cattle as 6.38 ± 0.51 minute.

Osbaldiston *et al.* (1970) reported higher BCT in Bovine (9.30 ± 0.6 minute) and equine (9.6 ± 1.4 minute) than Feline (5.2 ± 1.4 minutes) and canine (4.0 ± 0.3 minute).

Adwal and Gangwar (1971) made a detailed study on BCT in neonatal to adult buffaloes and found that the mean coagulation times was 11.13, 10.56, 12.3, 9.53, 9.34 and 9.4 minutes at the time of birth, 8-10 days, 14-15 days, 1-2 years 2-3 year and adult respectively.

Coles (1974) presented the value of BCT of different species of animals. It varied from 1-6 minutes, 3-15 minutes, 3.5-11.0 minutes, 2.5 minutes and 4.0 minutes respectively in Ovine, bovine, equine, porcine and canine.

Malik *et al.* (1974) found the Blood clotting time in male buffaloes 8.02 ± 0.388 minutes.

Schalm *et al.* (1975) stated that the erythrocyte sedimentation rate was rapid in equine, intermediate in dog, cat and pig and minimal in cow, sheep and goat.

Nockles *et al.* (1978) reported that sex and age significantly affected the blood clotting in immature cattle and sheep.

Kumar *et al.* (1991) observed no any significant change in clotting time of blood in normal cyclic, anestrous and repeat breeder buffaloes.

Shin *et al.* (1999) reported that ESR was unaffected in early pregnancy compared with normal values, but increased in the middle and late pregnancy. After parturition ESR returned to its normal value.

Prabha and Singh (2000) estimated the BCT in preparturient cows (4.12 ± 0.03 minute) significantly higher than calves at birth (3.12 ± 0.01 minute) and 1 month of age (3.27 ± 0.14 minute). It was similar to the BCT of 4-5 months (4.03 ± 0.08 minute) and 10-11 months (4.06 ± 0.01 minutes) calves. ESR of preparturient cows, calves at birth, at one month, at 4-5 months and 10-11 months were 14.13 ± 0.06, 8.16 ± 0.16, 11.06 ± 0.51, 18.30 ± 1.83 and 12.16 ± 0.06 mm / 24 hr. respectively.

2.3 Total Leucocytic Count and Differential Leucocytic Count

Mishra and Biswal (1961) reported the TLC in Orissa cattle as 9, 440 ± 2, 262 / mm^3 and Neutrophils, Eosinophils, Lymphocytes and Monocytes were 25 ± 2.87 %, 5 ± 1.52 %, 66 ± 2.05% and 4 ± 1.273% respectively.

Olbrich *et al.* (1971) reported the TLC, Monocyte, Lymphocyte, Neutrophil, Eosinophil and Basophils in Zebu (heat tolerant) cattle as 10.3 $\times 10^3$ / mm^3, 0.9%, 63.3%, 32.3%, 3.3% and 0.3% respectively. The same parameters in cold tolerant (scotch highland) were 12.1 $\times$ $10^3/mm^3$, 1.2%, 64.5% 21.4%, 12.6% and 0.2% respectively.

Schalm *et al.* (1975) found out the TLC and DLC in purebred Jersey female cattle and reported that in 3½ - 4½ months, 7½ - 9 months, 11-12 months, 15-19 months, 20-36 months, 3-4 years, 4-6 years and 6-14 years the TLC (thousand/mm^3) were 7.567, 8.000, 8.281, 8.840, 8.050, 7.063, 6.950 and 6.630 respectively ; neutrophil (%) were 28.2, 8.8, 12.9, 26.2, 24.0, 25.0, 21.0 and 18.5, lymphocytes (%) were 62.9, 82.2, 78.4, 63.0, 64.5, 60.5, 64.2 and 65.8, monocytes (%) were 8.0, 8.0, 6.9, 3.4, 5.0, 3.8, 5.0 and 3.2; Eosinophils (%) were 0.8, 1.0, 1.5, 6.6, 6.0, 9.7, 8.8 and 1.0 and Basophils (%) were 0.1, 0.0, 0.3, 0.8, 0.5, 1.0, 1.0, and 0.5% respectively.

Shukla *et al.* (1982) reported the TLC (thousand / mm^3) in dry, pregnant and lactating ewes as 8.80 $\pm$ 0.58, 9.04 $\pm$ 1.37 and 10.32 $\pm$ 1.69. In DLC neutrophil percentage was 37.8 $\pm$ 2.0, 31.1 $\pm$ 1.99 and 39.4 $\pm$ 4.73; eosinophil percent was 3.7 $\pm$ 0.56, 5.3 $\pm$ 1.39 and 3.4 $\pm$ 0.61; lymphocytes percent was 56.2 $\pm$ 2.23, 60.5 $\pm$ 2.24 and 53.9 $\pm$ 4.45 and monocytes percent were 3.2 $\pm$ 0.26, 3.1 $\pm$ 0.95 and 3.3 $\pm$ 0.67 respectively.

Sharma *et al.* (1985) studied the leucocytes in Murrah buffaloes of 0-3 months, 3-6 months, 6-12 months, 12-24 months, 2-4

years heifers and above 4 years adult buffaloes and reported TLC (thousand/mm^3) as 5.90 ± 0.10, 6.85 ± 0.15, 7.20 ± 0.20, 6.80 ± 0.11, 6.10 ±0.23 and 6.89 ± 0.30 ; Neutrophils (%) were 35.10 ± 3.50, 32.25 ± 4.50, 33.75 ± 6.38, 31.35 ± 5.50, 30.75 ± 4.86 and 28.70 ± 3.83, lymphocytes (%) were 50.90 ± 6.50, 54.15 ± 8.90, 55.25 ± 6.38, 57.50 ± 6.58, 57.25 ± 5.20 and 60.30 ± 3.55 (%) ; Eosinophils (%) were 7.50 ± 1.10, 6.35 ± 1.08, 4.50 ± 0.90, 2.50 ± 0.15, 5.50 ± 0.36 and 3.50 ± 0.15; Monocytes (%) were 6.50 ± 0.95, 6.25 ± 0.60, 6.00 ± 0.26, 8.50 ± 0.18, 6.50 ± 0.30 and 7.50 ± 0.31 and Basophils (%) were 0.00 ± 0.0, 1.00 ± 0.01, 0.50 ± 0.02, 0.15 ± 0.01, 0.00 ± 0.00 and 0.00 ± 0.00 respectively.

Misra and Prusty (1989) reported leucocyte count in 12-15 months jersey heifers and found that TLC was 12804±932 / mm^3 ; Neutrophil, Eosinophil, Basophil, lymphocytes and Monocytes were 17.26 ± 1.64%, 2.91±0.79%, 0%, 77.20 ± 1.63% and 2.82 ± 0.91% respectively.

Kumar *et al.* (1990) found out the leucocyte count in day old, 1 months, 6 months, 12 months, 20-24 months, 25-30 months and overall mean of female Murrah buffalo and reported that the TLC (thousand / mm^3) in above mentioned groups were 8.22 ± 1.49, 16.06 ± 1.06, 12.60 ± 1.35, 17.08 ± 3.54, 12.69 ± 1.65, 13.15 ± 1.52 and 13.30 ± 1.16 respectively. Neutrophil percent were 47.16 ± 3.30, 33.00 ± 1.63, 26.00 ± 6.67, 31.44 ± 3.48, 29.55 ± 1.99, 31.05 ± 3.06 and 33.03 ± 2.72, Lymphocytes counts (%) were 50.16 ± 3.69, 64.00 ±

1.69, 68.66 ± 6.40, 66.22 ± 3.63, 67.07 ± 1.99, 66.17 ± 3.66 and 63.71 ± 2.53, Eosinophils (%) were 2.00 ± 0.57, 2.00 ± 0.47, 2.30 ± 0.27, 1.44 ± 0.27, 2.30 ± 0.40, 2.42 ± 0.38 and 2.07 ± 0.13, and monocytes (%) were 2.00 ± 0.05, 1.00 ± 0.47, 2.00 ± 0.06, 0.11 ± 0.10, 0.98 ± 0.20, 1.09 ± 0.12 and 1.91 ± 0.27 in the respective age groups.

Kumar *et al.* (1991) reported the TLC (thousand / mm^3 of blood) in normal cycling, anoestrus and repeat breeder non descript rural buffaloes as 6.18 ± 0.73, 9.57 ± 1.47, 8.61 ± 1.21 respectively.

Gupta *et al.* (1996) studied the different leucocyte count in different physiological status of Sahiwal cows. TLC (per mm^3) in calves below 6months, heifer of 1-2 year, 3-6 months pregnant, more than 7 months pregnant, cows after parturition, milking cows and non milking cows were 6970.9 ± 296.75, 7594.8 ± 238.25, 6233.6 ± 345.92, 6346.0 ± 411.39, 6718.2 ± 214.74, 6787.0 ± 157.34 and 7802.0 ± 75.14 respectively. In the same animals Neutrophil (%) were 13.2 ± 1.65, 20.6 ± 1.97, 8.8 ± 1.97, 6.0 ± 0.9, 14.2 ± 1.35, 6.4 ± 0.73 and 16.6 ± 0.78 respectively, Basophils (%) were 0.8 ± 0.33, 0.2 ± 0.18, 0.4 ± 0.22, 0.6 ± 0.36, 0.6 ± 0.33, 0.8 ± 0.36 and 0.6 ± 0.36; Eosinophils (%) were 6.2 ± 1.14, 7.6 ± 0.61, 7.0 ± 0.63, 6.8 ± 0.52, 8.2 ± 0.52, 6.6 ± 0.47 and 12.2 ± 0.77 lymphocytes (%) were 71.4 ± 2.73, 63.6 ± 0.83, 75.4 ± 0.92, 81.0 ± 1.52, 68.8 ± 0.45, 77.6 ± 0.79 and 60.6 ± 2.06 and monocytes (%) were 8.4 ± 0.45, 8.6±0.45, 8.2 ± 0.33, 5.2 ± 0.9, 9.8 ± 0.71 and 8.6 ± 0.21 and 10.0 ± 0.75 respectively.

Mehere *et al.* (2002) reported the TLC in crossbred cows of 4 weeks prepartum, 3 weeks prepartum, 2 weeks prepartum, one week prepartum, on the day of parturition, one week post partum, two weeks postpartum, 3 weeks postpartum and 4 weeks post partum as 10.15 ± 0.63, 9.90 ± 0.46, 9.78 ± 0.64, 11.89 ± 1.38, 8.93 ± 0.68, 7.90 ± 0.66, 10.57 ± 1.07, 11.77 ± 1.59 and 10.48 ± 0.85 thousand / mm^3.

2.4 Total Serum Protein, Albumin and Globulin

Olbrich *et al.* (1971) reported the mean TSP level in heat tolerant (zebu) and cold tolerant (Scotch highland) as 7.0 g/100ml and 8.1 g/100 ml respectively.

Malik *et al.* (1974) reported the serum total protein in 9-12 months buffalo calves as 6.30 ± 0.156 g/100 ml. Albumin and Globulin level were 3.64 ± 0.917 g/100ml and 2.66 ± 0.152 g/100 ml respectively.

Payne and Maitra (1980) reported the TSP level (g/100ml) in 16-20 months heifer of Hariana, Sahiwal and Gir breed as 7.45, 7.45 and 7.61 respectively.

Roussel *et al.* (1982) reported the total serum protein (g/100ml) in Jersey cow at 1 years, 3 years, 4 years, 5 years, and 6 years which was 6.19 ± 0.50, 6.51 ± 0.50, 7.04 ± 0.57, 7.10 ± 0.62, 7.03 ± 0.23 and 7.74 ± 0.5. In the same animal the globulin level (g/100ml) was 2.82 ± 0.52, 3.36 ± 0.59, 3.76 ± 0.76, 4.06 ± 0.91, 4.63 ± 0.32 and 5.28 ± 0.86 respectively.

Kulkarni *et al.* (1983) stated the TSP level in 5-8 years old Gir and Gir × HF lactating cows as 6.37 ± 0.00 g% and 6.53 ± 0.01g% ; albumin level was 2.85 ± 0.00 g% and 2.89 ± 0.29 g% and globulin was 3.51 ± .0.12 g% and 3.46 ± 0.03 g% respectively.

Kavani *et al.* (1987) reported that the TSP in normal cyclic heifers was significantly higher (7.14 ± 0.22 g(%)) than infertile heifers (5.65 ± 0.23 g%). Albumin level was significantly higher in cycling heifers (3.98 ± 0.10 g%) than in infertile heifers (3.47 ± 0.06 g%). Globulin level was non-significantly higher in cyclic heifers (3.20 ± 0.13 g%) than infertile heifers (2.61 ± 0.22 g%).

Dutta *et al.* (1988) reported that the total serum protein concentration in normal cycling jersey heifers (8.76 ± 0.30 g/dl) was significantly higher than that of anoestrous jersey heifers (6.58 ± 0.42 g/dl).

Aminlari *et al.* (1989) from Iran reported the TSP (g/100ml) in female Sistani breed cattle of 1-2 year, 2-3 year, 3-4 year, 4-5 years and 5-6 years as 6.3±1.0 5.3 ± 1.2, 5.9 ± 1.0, 5.8 ± 1.2 and 5.8 ± 1.4 g/100 . In the same animals albumin level (g/100ml) was 3.0 ± 0.04, 3.1 ± 0.6, 2.8 ± 0.5, 2.5 ± 0.5 and 2.4 ± 0.5 and globulin (g/100ml) was 3.3 ± 1.0, 2.2 ± 1.0, 3.1± 0.9, 3.2 ± 1.3 and 3.4 ± 1.4 respectively.

Gujar *et al.* (1990) reported the mean serum protein at fertile and non fertile estruses as 7.59 ± 0.09 g/100ml and 6.34 ± 0.12 g/100 ml respectively.

Ali *et al.* (1991) observed the level of TSP in crossbred (Jersey × non descript) of 18-24 months cyclic heifer and 36 months non – cycling heifers and found as 7.28 ± 0.36 g% and 4.46 ± 0.12 g%.

Gaikwad *et al.* (1992) reported the TSP level (g/dl) in Jersey × Red Khandhari cattle in 0-6 months, 6-12 months, 24-42 months heifers and above 48 months cows as 6.37 ± 0.08, 6.19 ± 0.10, 6.95 ± 0.09 and 7.89 ± 0.020. Albumin level (g/dl) in the same animals were 2.27 ± 0.06, 2.27 ± 0.08, 2.35 ± 0.08 and 2.35 ± 0.03 while the Globulin level (g/dl) was 4.09 ± 0.12, 3.91 ± 0.08, 4.57 ± 0.13, and 5.53 ± 0.22 respectively.

Gandotra *et al.* (1993) found the value of total serum protein (g/100ml) in repeat breeder cattle (9.31 ± 0.97) and buffaloes (10.7 ± 0.55) which did not vary significantly from that of normal cattle (10.0 ± 0.48) and buffaloes (10.8 ± 0.77).

Sharma *et al.* (1994) observed the value in 0-6 months non descript indigenous and crossbred jersey calves. TSP level in female indigenous and cross bred Jersey was 5.71 ± 0.17 g/100ml and 5.39 ± 0.08 g / 100 ml. Albumin was 3.43 ± 0.07 g/100ml and 3.41 ± 0.08 g/100ml and globulin was 2.28 ± 0.13 g/100ml and 1.98 ± 0.05 g/100ml respectively.

Shrivastava and Kadu (1995) reported that the TSP level was significantly higher in normal cycling (7.54 ± 0.11 g%) than in delayed pubertal (6.64 ± 0.26 g%) crossbred heifers.

Vhora *et al.* (1995) reported that serum total protein were significantly higher in normal cycling cows (8.62 ± 0.13 g/100 ml) than in anestrous cows (6.82 ± 0.40 g/dl).

Ramakrishna (1997) estimated the total protein, albumin and globulin level in jersey crossbred anoestrus cows as 5.91 ± 0.398 g/100ml, 2.88 ± 0.101g/100ml and 3.15 ± 0.169 g/100 ml. The same constituents in cycling cows were 6.85 ± 0.168 g/100ml 3.06 ± 0.43 g/100ml and 3.34 ± 0.16 g/100 ml respectively.

Tandle *et al.* (1997) reported the TSP in non-descript cows between 5-7 years which was significantly higher (7.74 ± 0.37 g%) in oestrus cows than non oestrous cows (4.41 ± 0.27 g%).

Arosh *et al.* (1998) reported that the normal cyclical cows had significantly higher level of total serum protein, albumin and globulin than that of anestrus cows. These were 7.45 ± 0.39 and 4.80 ± 0.53 mg%, 3.65 ± 0.25 and 3.11 ± 0.29 mg% and 3.80 ± 0.16 and 1.69 ± 0.47 mg% respectively in cycling and non cycling cows.

Sharma *et al.* (1998) reported the significantly higher plasma protein (7.28 ± 0.20 mg/dl) in subestrous heifers than in normal cyclic (6.65 ± 0.06 mg/dl) and anestrous heifers (6.37±0.04 mg/dl).

Srikhande and Sarode (1999) reported the TSP (g/dl) in cows below 5 years, 5-8 years and above 8 years as 7.04 ± 0.10,7.25 ± 0.11 and 7.17 ± 0.08. Albumin (g/dl) value was 3.32 ± 0.09, 3.37 ±

0.09 and 3.06 ± 0.10 g/dl. Globulin (g/dl) value was 3.72 ± 0.09, 3.83 ± 0.10 and 4.11 ± 0.06 g/dl.

Patil *et al.* (2000) found the protein level in 0-3 months, 3-6 months, 6-9 months, 9-12 months, 12-15 months and 15-18 months Gir and Gir × HF females. TSP level (g/100ml) in Gir and Gir × HF in different age group were 5.32 ± 0.21 and 5.62 ± 0.21; 5.87 ± 0.06 and 6.20 ± 0.16 ; 6.08 ± 0.13 and 6.48 ± 0.17; 6.62 ± 0.32 and 6.58 ± 0.11; 6.77 ± 0.11 and 6.67 ± 0.14 and 6.82 ± 0.06 and 6.80 ± 0.24 respectively. Albumin level (g/100ml) in the same animals were 3.52 ± 0.15 and 3.37 ± 0.22 ; 3.62 ± 0.14 and 3.88 ± 0.04; 3.58 ± 0.01 and 3.70 ± 0.13; 3.53 ± 0.10 and 3.63 ± 0.09; 3.60 ± 0.22 and 3.53 ± 0.10 and 3.55 ±0.10 and 3.77 ± 0.09. Globulin level (g/100ml) in the same animals were 1.80 ± 0.19 and 2.27 ± 0.11; 2.25 ± 0.18 and 2.32 ± 0.18; 2.50 ± 0.18 and 2.78 ± 0.14; 3.08 ± 0.32 and 2.95 ± 0.14; 3.17 ± 0.24 and 3.13 ± 0.19 and 3.21 ± 0.18 and 3.03 ± 0.25 respectively.

Kabir *et al.* (2001) observed the serum protein in cyclic and acyclic buffaloes and found as 8.46 ± 0.11 g/dl and 7.92 ± 0.11 g/dl respectively.

Kumar *et al.* (2001) studied the TSP level (g/dl) in advanced pregnancy, 1 week postpartum and 2 months post partum (peak yield) and reported as 10.33 ± 0.71, 9.35 ± 0.26 and 8.64 ± 0.48 respectively in cows and 10.18 ± 0.59, 9.06 ± 0.45 and 8.49 ± 0.91 respectively in buffaloes.

2.5 Uric Acid and Creatinine

Olbrich *et al.* (1971) reported the creatinine level in heat tolerant (Zebu) and cold tolerant (Scotch Highland) heifers as 2.07 g/100ml and 1.97 mg/100 ml serum.

Berglund and Oltner (1983) reported that the mean creatinine concentration increases from 80 μ mol/lit at 3-6 months of age to 129 μ mol/lit at 2 year in dairy heifers.

Kulkarni *et al.* (1983) observed the creatinine level in 5-8 years old Gir and Gir × HF lactating cows as 1.14 ± 0.00 mg% and 1.13 ± 0.01 mg% respectively.

Kulkarni *et al.* (1984) reported the creatinine level in Gir and Jersey lactating cows aging between 4-5½ years as 1.20±0.03 mg/dl and 0.99± 0.02 mg/dl respectively.

Kulkarni *et al.* (1984a) estimated the creatinine level in 4-10 years old lactating and dry Murrah buffaloes as 1.46±0.04 and 1.56±0.08 mg% respectively. The value was statistically not different.

Meli *et al.* (1984) reported that value for uric acid was lower in repeater cows than the normal fertile cows.

Sharma *et al.* (1994) studied the level in 0-6 months non descript indigenous and crossbred Jersey female calves and reported the uric acid level (mg/dl) 6.30 ± 0.22 and 5.53 ± 0.38 while the creatinine (mg/dl) was 0.96 ± 0.10 and 1.19 ± 0.06 in respective groups.

Kokilaprabhakaran *et al.* (1997) estimated the creatinine concentration in 3 – 5 months pregnant jersey heifer 0.24 ± 0.04 mg%.

Arosh *et al.* (1998) reported the creatinine level in cyclical and anoestrus cows as 0.58 ± 0.10 mg% and 0.56 ± 0.50 mg% which were differing non significantly.

Kalita and Mahapatra (1999) reported the level of uric acid in Black Bengal kids on 90 days, 105 days, 120 days, 135 days and 150 days and found the value (mg/dl) as 1.158 ± 0.092, 1.130 ± 0.081, 1.483 ± 0.076, 1.788 ± 0.192 and 1.828 ± 0.140 respectively. The creatinine (mg/dl) level in the same kids were 0.907 ± 0.084, 1.050 ± 0.061, 1.170 ± 0.102, 1.185 ± 0.666 and 1.317 ± 0.104 respectively.

Patil *et al.* (2000) measured the level in healthy female calves of Gir and Gir × H.F. of different ages. The uric acid level (mg/dl) in 0-3 months, 3-6 months, 6-9 month, 9-12 months 12-15 month and 15-18 months of Gir and crossbred calves were 1.95±0.30 and 1.57±0.24; 1.58±0.18 and 1.60 ± 0.19; 1.58 ±0.13 and 1.12±0.16; 1.50±0.09 and 1.22±0.14; 1.63±0.11 and 1.38±0.19 and 1.40±0.18 and 1.58±0.14 mg/dl respectively. The creatinine level (mg/dl) in the same animals were 0.78±0.11 and 0.85±0.11; 0.95 ±0.06 and 0.92±0.07; 1.12±0.06 and 1.07±0.04 ; 1.17 ± 0.11 and 1.05 ± 0.04; 1.32 ± 0.04 and 1.07 ± 0.09 and 1.30±0.08 and 1.15±0.02 mg /dl respectively.

Kumar and Pachauri (2001) estimated the creatinine level in 12-15 months old heifers, 4–6 months pregnant heifer, 5 – 8

years empty dry cows, 4-6 months pregnant lactating cows, lactating cows between 4 – 8 week of lactation yielding 8–12 liter milk daily. In summer (May – June) the value (μmol/lit) was 316.03, 223.21, 221.00, 203.32, 280.67, 227.63 while in winter (Dec-Jan.) the level (μmol/lit) was 102.54, 90.61, 133.04, 101.57, 127.12 and 95.03 respectively.

2.6 Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT)

Chhabra and Mehta (1967) found the mean SGOT level in Malvi cow calves, Murrah Buffalo and indigenous goats as 31.2 49.3 and 48.9 unit respectively. SGPT activity in the same cow calves was 14.2 units. No measurable SGPT activities were noted in Buffalo calves and goats.

Olbrich *et al.* (1971) reported the level of SGOT in Zebu and Scotch Highland breed cattle as 96.0 mu/ml and 130.0 mu/ml.

Samanna and Ramaswamy (1976) studied the normal level in cattle in Madras and reported the range from 6.5 to 26.5 units (mean 14.14) for SGPT and 13 to 52 Units (Mean 28.40) for SGOT.

Murtuza *et al.* (1980) studied the female Hariana cattle in various physiological states and reported that SGOT activity (M.I.U.) was 38.85 ± 1.76, 39.60 ± 3.79, 35.85 ± 1.62 and 45.82±0.83 in heifers, empty dry cows, late pregnant cows and early lactating cows respectively while the SGPT activity (M.I.U.) in the same groups were 19.20 ± 1.46, 16.13 ± 1.15, 21.02 ± 1.50 and 26.97 ± 3.72 respectively.

Payne and Maitra (1980) found the SGOT level (units /ml) 49.52, 48;15 and 55.25 in 16-20 months heifers of Hariana, Sahiwal and Gir respectively. In the same group SGPT activity (units / ml) was reported as 17.65, 17.85 and 18.15 respectively.

Roussel *et al.* (1982) studied aspartate transaminase level in different aged jersey cows and could not found any significant fluctuation. The value (nkat/L) in 1 year, 2 years, 3years, 4 years, 5 years and 6 years, old cows were 1800.36 ± 674.97 1,718.51±561.11, 2,080.58 ± 738.65. 2,260.29, ±738.48, 1,778.19 ±254.72 and 2,000.40 ± 636.96 respectively.

Sharma *et al.* (1986) reported that SGOT level (µmol / min/lit) was higher (32.36±5.35) in primary infertile heifers than normal cyclic heifers (30.34±5.38) but the difference was non significant. The SGPT level (µmol / min/ lit) was significantly lower in infertile heifers (30.23 ±4.70) than normal heifers (65.29 ± 13.98).

Kataria *et al.* (1991) found that the transaminasa activity in camels vary significantly according to age. In the age group below 4 years, between 4-10 years and above 10 years the AST level (RF unit / ml) was 94.11 ± 1.48, 76.66 ± 1.27 and 68.49 ± 1.85 while in the same group ALT (RF units / ml) was 12.69±0.43, 9.48±0.25 and 7.54±0.15 respectively.

Pal *et al.* (1991) reported the level of SGOT and SGPT in the cycling heifers and cows which were significantly higher than non cycling ones.

Behera *et al.* (1993) estimated the transaminase activity in female Black Bengal goats of different ages. They reported the SGOT level (IU/100 ml) in female of 3 months as 5.45 ±0.14, in 6 months 4.56±0.20, in 9 months 5.73±0.12, in 12 months 4.34±0.39 and in 24 months and above it was 4.25 ± 0.10. The SGPT level (IU/100 ml) in the same animals were 1.46 ±0.15, 1.12±0.11, 1.21±0.10, 1.22±0.23 and 1.13±0.06 respectively.

Gandotra *et al.* (1993) reported the values (nmol pyruvate formed /min/ml) of alanine aminotransferase as 42.5 ± 8.52 and 32.2 ± 8.80 in normal and repeat breeder cattle; 34.3±0.59 and 33.6 ± 2.96 in normal and repeat breeder buffaloes respectively. The value of aspartate aminotransferase (nmol Pyruvate formed/ min/ml) in normal and repeat breeder cows were 14.2 ± 1.95 and 21.5 ± 5.58 and in normal and repeat breeder buffalo it was 22.7 ± 2.48 and 34.0±4.39 respectively.

Kokilaprabhakaran *et al.* (1997) found the SGOT and SGPT level in pregnant jersey heifer (3-5 months pregnant) as 78 ± 6 and 27±8 IU/L respectively.

Arosh *et al.* (1998) estimated significantly low level of SGOT in anoestrus cows (67.00 ± 4.09 IU) than in normal cyclic cows (107.83 ± 5.64 IU).

Sharma *et al.* (1998) estimated the SGOT and SGPT activities significantly higher in anestrus buffalo heifers (145.58 ± 5.51 U/L 83.77 ± 2.36 U/L) as compared to normal cyclic buffalo heifers (145.58 ± 5.51 U/L and 68.30 ± 6.38 U/L).

Atak *et al.* (2000) studied the SGOT and SGPT level in different age group of Gir and crossbred female calves and reported the SGOT level (IU/L) in 0-3 months, 3-6 months, 6-9 months, 9-12 months, 12-15 months and 15-18 months old calves as 92.28± 8.05, and 61.13 ± 3.30; 82.15 ± 3.19 and 80.31 ± 1.14, 68.78 ± 5.82 and 73.21 ± 6.53, 68.35 ± 3.80 and 75.85 ± 4.64, 59.55± 1.81 and 88.65 ± 5.14 and 63.91 ± 7.12 and 78.08 ± 2.83 respectively The SGPT level (IU/L) in Gir and crossbred female calves of the same age group were 24.11 ± 1.77 and 22.60 ± 3.19 26.86 ± 2.56 and 20.70 ± 2.86, 28.21 ± 2.66 and 32.70 ± 4.21, 29.58±2.12 and 28.53 ± 4.21, 30.31±2.01 and 25.15 ± 4.33 and 28.00 ± 4.10 and 25.23 ± 3.53 respectively.

Rathee *et al.* (2002) studied the transminase activities in female Murrah buffalo calves aging 1-3 days, 4-30 days, 3-6 months, 7-12 months, 18-24 months heifers, 25-30 months heifers and 31-36 months heifers from CIRB, Hisar and reported that the AST (IU/L) level was 154.35±2.14, 147.26±09.88, 163.80±6.22, 160.50±14.50, 157.70±11.50, 156.15±13.86 and 173.90±12.76 while the ALT level (IU/L) was 22.97± 2.39 19.27± 127, 42.36±3.12, 49.82±3.17, 57.36±4.07, 63.27±4.91 and 72.51±3.36 respectively.

2.7. Alkaline Phosphatase

Goswami *et al.* (1971) found the level in young and adult Hariana cattle (included both male and female) as 7.37 ± 0.52 units and 9.33 ± 0.47 units respectively.

Olbrich *et al.* (1971) measured the level in heat tolerant (zebu) and cold tolerant (Scotch highland) cattle maintained under identical environment and found as 653 and 266 mu/ml respectively.

Singh *et al.* (1973) studied the level (IU/L) in cows and heifers and reported 4.325±0.670 and 5.275±0.170 respectively in Rathi. In Sahiwal the value were 2.700±0.140 and 4.030±0.200 in respective group.

Pandiya *et al.* (1977) studied the level in different aged dairy cattle (included Rathi Sahiwal. and their crosses with Red Dane, Brown Swiss and Jersey) and found as 3.15 ±0.221, 3.20±0.268, 3.63±0.151, 4.25±0.149, 4.82±0.136, 3.73±0.273 and 3.19±0.223 Bodansky units/ 100 ml respectively in 0-6, 6-12, 12-18, 18-24, 24-30, 30-36 and above 36 months.

Murtuza *et al.* (1980) found the level in Heifers, empty dry cows, late pregnant cow and early lactating cows as 3.29±0.23, 2.87 ± 0.36, 2.63±0.20 and 1.47±0.21 Bodansky units/ 100 ml respectively.

Roussel *et al.* (1982) reported the level (n Kat/ L) in Jersey heifer of 1 year, Jersey cows of 2 year, 3 years, 4year, 5 year and 6 year as 618.12±233.88, 431.92±185.04, 276.22±139.36, 233.38±100.35, 266.72±125.86 and 169.70±53.18 respectively.

Mazumder and Mazumder (1985) estimated the level of alkaline phosphatase activity in different F_1 crossbreds and reported the level (KA units/100ml) in male and female of Friesian × Hariana as 14.29 ± 1.50 and 19.18 ± 1.81; in Jersey x Hariana as 21.42±3.26 and 21.44 ± 1.06; in Brown Swiss × Hariana as 20.93 ± 4.49 and 18.43 ± 0.91 respectively. In an other experiment they found that the age has significant effects on the ALP activities. In Friesian × Hariana the level (KA units / 100 ml serum) was 19.82±3.44, 21.07±1.40, 20.49±1.63, 19.58±2.21, 17.16±1.46 and 12.93±1.35 in 9, 12, 24 and 36 months respectively.

Sharma *et al.* (1986) estimated the level in normal and primary infertile heifers as 4.98±0.75 B.L. units/ ml and 7.37±0.45 B. L. units/ ml respectively

Gandotra *et al.* (1993) reported the value (nmol phenol produced/ min/ml serum) in normal cycling and repeat breeder cattle as 52.6 ± 0.27 and 60.3 ± 10.1 respectively, while in normal cycling and repeat breeder buffaloes the value was 107.4 ± 17.0 and 80.5 ± 16.5 respectively.

Behera *et al.* (1993) reported the level (IU/dl) in Black Bengal female goats of 3, 6, 9,12 and 24 months 3.06±0.26, 4.77±0.54 4.65±031, 3.11±0.18 and 4.14±035 respectively.

Sharma *et al.* (1994) studied the activity in 0-6 months calves of either sex in non descript indigenous and crossbred jersey.

The value was 57.5 ± 1.74 and 47.1±3.25 IU/Lit/min in indigenous and crossbred male calves while in female indigenous and crossbred calves value was 55.9±2.20 and 44.7±3.33 IU/lit/min. respectively.

Kokilaprabhakaran *et al.* (1997) reported the level in 3-5 months pregnant jersey heifers as 32.4 ±1.2 IU/L.

Atak *et al.* (2000) observed the alkaline phosphatase activities in different aged Gir and crossbred female calves and reported that in 0-3 months, 3-6 months, 6-9 months, 9-12 months, 12-15 months and 15-18 months the level (IU/L) was 309.33± 15.95, 350.60±77.60, 264.40±26.85, 213.68±25.13, 197.38±36.17 and 242.95±31.95 for Gir and for crossbred calves it was 283.33±43.46, 230.28±66.46, 126.23±9.95, 192.91±17.17, 187.91±1925 and 137.91 ± 10.12 IU/L respectively.

2.8 Sodium and Potassium :

Olbrich *et al.* (1971) reported the serum sodium level in heat tolerant (zebu) and cold tolerant (Scotch highland) cattle as 151.0 and 148.2 mEq/lit. Potassium level in the same animal was 4.4 and 4.5 mEq/lit. respectively.

Agarwal *et al.* (1982) reported sodium and Potassium level in normal and repeat breeding jersey sahiwal crossbred cows on 1 day, 13th day and 16th day of estrous. The sodium level (mEq/lit) in normal and repeater was 104.80 ± 2.57 and 117.06 ± 9.73; 108.00±4.98 and 113.13 ± 4.64 and 101.60 ± 4.47 and 105.56 ±2.33.

while the potassium level (mEq/lit) were 3.40 ± 0.17 and 3.53 ± 0.06; 3.10±0.11 and 3.09 ± 0.20 and 3.26 ± 0.13 and 3.31 ± 0.07 respectively. On the same day of estrous the sodium level (mEq/lit) in Murrah buffaloes was 117.60 ± 2.93 and 107.50 ± 3.27; 124.20 ± 4.36 and 102.50 ± 2.73 and 3.60±0.19 and 3.49±0.22 while the Potassium level (mEq/lit) was 3.26 ± 0.30 and 3.99 ± 0.63 3.10 ± 0.14 and 3.12 ± 0.11 and 4.30 ± 0.20 and 4.00 ± 0.22 respectively.

Borvonsin and Sirikhajornbhandhu (1983) reported the sodium and potassium level in 10-19 months old heifers as 139.17 ± 0.93 mEq/lit and 5.40 ± 0.09 mEq/lit plasma respectively.

Kulkarni *et al.* (1983) observed 5-8 years old Gir and Gir × HF lactating cows and reported the sodium level (mEq/ it) as 139.73 ± 1.21 and 143.43 ± 0.11 and potassium level as 5.74 ± 0.09 and 5.04 ± 0.09 mEq / L respectively.

Kulkarni *et al.* (1984) reported the sodium level (m Eq/lit) in 4-5 ½ years old Gir and Jersey cows as 141.12 ± 1.34 and 134.70 ± 1.68 respectively while the potassium level was 4.82 ± 0.12 and 5.11 ± 0.10 respectively.

Kulkarni *et al.* (1984a) reported the sodium level in lactating and dry Murrah buffalo as 144.00 ± 1.32 mEq/L and 148.10 ± 3.83 mEq./ L. The potassium level in the same animal was 4.59 ± 0.08 mEq/lit and 4.97 ± 0.22 mEq/Lit respectively.

Kumar *et al.* (1986) reported the sodium level (mEq/lit) in normal cyclic, anoestrus and repeat breeders cows 142.84 ± 1.39, 124.79 ± 2.66 and 127.25 ± 1.83. while the potassium level (mEq/lit) was 4.93 ± 0.07, 4.87 ± 0.10 and 4.17 ± 0.07. In the case of cyclic, anoestrus and repeat breeder heifers the sodium level (mEq/lit) was 138.05 ± 2.58, 131.33 ± 2.41 and 131.22 ± 2.15. while the potassium level (mEq/lit) was 5.00 ± 0.11, 4.17 ± 0.12 and 4.67 +0.10.

Barua *et al.* (1988) reported the sodium level in 3-7 year old jersey cows on 5^{th}, 10^{th}, 15^{th} and 20^{th} day of estrous cycle as 139.13± 2.28, 143.13 ± 2.18, 141.63 ± 2.44, 139.88 ± 2.72 and 143.63 ± 1.89 mEq/L while the potassium level in the same animals were 5.23 ± 0.54, 5.08 ± 0.43, 5.28 ± 0.47, 4.53 ± 0.43 and 5.48 ± 0.75 mEq/L respectively.

Sharma *et al.* (1995) reported the sodium level (mEq/lit) in non pregnant 3-4 years old non descript Kashmiri and Jersey cows as 149.26 ± 7.32 and 126.36 ± 8.26 while the potassium level (mEq/lit) was 4.78 ± 0.32 and 3.09 ± 0.38 respectively.

Arosh *et al.* (1998) reported that the sodium level in normal cycling cows (83.33 ± 2.88) mEq/ml) was higher than anestrus cows (63.50 ± 2.35 mEq/ml). Potassium level was also significantly higher in cycling (2.55 ± 0.25) mEq/ml) cows than in non cycling cows (1.91 ± 0.24 mEq/ml).

Shrikhande and Sarode (1999) reported sodium level (mEq/lit) in cows below 5 year, 5-8 years and above 8 year as 134-07 ± 1.20, 133.33 ± 1.16 and 132.95 ± 1.44 while the potassium level (mEq/lit) was 5.00 ± 0.20, 4.96 ± 0.16 and 4.98 ± 0.13 mEq / lit respectively.

Singh *et al.* (1999) reported the sodium level (mEq/lit) in anoestrus normal cyclic, post partum, 3 months pregnant, 3-6 months pregnant and 6-9 months pregnant yak as 135.50 ± 9.39, 137.0 ± 9.34, 149.42 ± 4.31, 133.25 ± 9.33, 128.75 ± 8.38 and 154.00 ± 3.96 mEq./lit. In the same animal potassium (mEq/lit) was 5.37 ± 0.67, 4.30 ± 0.28, 4.77 ± 0.14, 3.92 ± 0.25, 5.00 ± 0.67 and 4.94 ± 0.15 mEq/lit respectively.

Kumar *et al.* (2001) reported the sodium level (mg/dl) in pregnant, within 1 week postpartum and around 2 months postpartum cows as 247.0 ± 3.18, 236.0 ± 2.88 and 232.56 ± 450. In the same animal potassium level (mg/dl) was 29.70 ± 1.34, 31.80 ± 0.77 and 35.0 ± 1.26 gm/dl. In buffaloes in the same group sodium level (mg/dl) was 247.91 ± 2.91, 238.33 ± 4.61 and 235.41 ± 7.13 mg/dl, while Potassium level (mg/dl) was 31.20 ± 1.59, 32.30 ± 1.30 and 35.30 ± 1.67 respectively.

Chapter – 3

Materials and Methods

MATERIALS AND METHODS

3.1 Experimental Animals :

The experimental animals for present investigation were selected from animal herd of Animal Production Research Institute (APRI), RAU, Pusa, Samastipur. Six noncycling crossbred heifers (Friesian 75% × Hariana 25%) in each group of 10-13 months, 18-21 months and 27-30 months were selected. Another six cycling crossbred heifers (of the same genetic composition) and six cycling lactating crossbred cows (75% HF × 25% Hariana) were also selected from the same herd. All the animals were maintained in the animal herd under general herd managemental condition. The genitalia of all the animals were free from ecto and endoparasites. Their rectal temperature, respiration rate, pulse rate and ruminal motility were within the normal range.

All the experimental animals were apparently healthy and were maintained in the herd of the respective age group. They were being offered *adlib.* feeds and fodders in the herd as was practiced in the farm twice daily, morning (5.00 am to 6 am) and evening (3.00 p.m. to 4 p.m.). Fresh drinking water was made available to the animals throughout the day and night during the experiment.

3.2 Collection of Blood Samples and its preservation :

Blood samples from each experimental animal were collected during fully conscious state. Attempts were made to avoid stress and strain to the animals during blood collection. Blood samples (25 ml) from each animals were collected by venipuncture from jugular vein using sterilized hypodermic needle (18 gauze) between 6 a.m. to 7 a.m. Single blood sample from each animal was collected. Blood samples were processed after collection for blood clotting time, hemoglobin concentration, total erythrocyte count, total leucocyte count and Differential Leucoccyte count. About 5 ml blood from each animal was taken in a vial having anticoagulant (sodium citrate) for the estimation of packed cell volume (PCV) and Erythrocytic Sedimentation Rate (ESR). About 20 ml of blood was kept in glass test tube in slanting position at room temperature for separation of serum. After 2 hours, the tubes having clotted blood was centrifuged at 3000 rpm for 10 minutes and the supernatant clear serum was separated from the clot by means of a glass Pasteur pipette, divided into different aliquots and stored in deep freeze in sterile plastic vials. Estimation of total serum protein, serum albumin, globulin, uric acid, creatinine, SGOT, SGPT, alkaline phosphatase, sodium and potassium was done from the blood serum.

3.3 Blood Clotting Time (BCT)

Blood clotting time was estimated as described by Schalm *et al.* (1975) as follows :

The capillary tubes (8 cm length and 0.8 to 1.22 mm diameter) were taken and filled with freshly collected venous blood upto $3/4^{th}$ of its length. Holding the tube between the thumb and index finger the tube was broken gently at 15 seconds interval until a strand of fibrin was seen extending across the gap between the broken ends of the tube. The time between the appearance of blood in the syringe and appearance of fibrin strand was considered as the clotting time.

3.4 Hemoglobin (Hb) estimation

Hemoglobin was estimated as described by Kolmer *et al.* (1969) using Sahli's hemometer as follows :

N/10 HCl was placed in graduated glass tube upto the mark twenty. 0.02 ml of freshly collected venous blood was taken by Sahli pipette and was transferred into the graduated tube. Pipette was rinsed by drawing blood mixed with N/10 HCl into its, twice. The tube with acid blood was kept aside for ten minutes. When the full reddish brown colour of the acid hematin (Hemin) was formed, the content of the tube was carefully diluted with glass distilled water until the colour exactly matched with the standard colour prism fitted in the comparator. The Hb in gram /100 ml in the blood was recorded as per the graduation marked on the tube.

3.5 Total Erythrocyte Count (TEC)

The total erythrocyte count was done by the method as described by Schalm *et al.* (1975) as follow :-

Freshly collected venous blood was drawn in red cell pipette upto '0.5' mark and the tip of the pipette was wiped clean. The blood was diluted by drawing the physiologic solution (sodium chloride – 0.85 g and Distilled water – 100 ml) to the '101' mark, thus making a dilution of 1 : 200. The pipette stem was rolled back and forth between the thumb and index finger for two minutes for proper mixing. After this the counting chamber was charged with well mixed blood and diluting fluid and left for about three minutes to settle the erythrocytes in single level. The number of cells in five secondary squares (4 corner squares and one central square) were counted with high dry objective of the microscope. This number was multiplied by 10,000 for getting the total erythrocyte number per µl of blood.

3.6 Total Leukocytic Count (TLC)

TLC was done by the method as described by Schalm *et al.* (1975), as follow :-

Freshly collected venous blood was drawn to the mark '0.5' of white cell pipette and the tip of the pipette was wiped clean. Then the diluting fluid [Glacial acetic acid – 2.0 ml, Distilled water – 98.0 ml and Gentian violet (1% aqueous solution) – 1.0 ml] was drawn to the mark '11', thus, making a dilution of 1 : 20. The pipette was rotated between thumb and index finger for two minutes to facilitate proper mixing. After this the chamber was charged and left for 3 minutes to settle the cell in single plane. The cells in the four corner blocks (Primary Squares) were counted with low power objective of the microscope. To obtain the total leukocytic count/ µl of undiluted blood, this value was multiplied by 50.

3.7 Differential Leukocytic Count (DLC)

The DLC was done by the method as described by Schalm *et al.* (1975) as follows :-

Chemical – Giemsa stain

Giemsa powder – 1.0 gm, Glycerol – 66.0 ml

Methanol – 66.0 ml

3.7.1 Preparation of Giemsa stain :

1 gram Giemsa powder was placed in a glass mortar and 5 ml of glycerol was added to it and it was ground properly to dissolve the Giemsa stain. The remaining amount of glycerol was then added to the mixture. The whole mixture was transferred into glass bottle and kept at 60^0 C for 30 minutes. After this incubation 66 ml methanol was added to the mixture and kept at room temperature. At the time of staining the Giemsa stain was diluted 1 : 10 with glass distilled water (1 part Giemsa stain and 10 parts distilled water).

3.7.2 Preparation of Blood Smear and It's Staining :

The blood film was prepared on clean dry glass slide with freshly collected venous blood from each animal. The blood film was fixed by placing the blood film in absolute methyl alcohol for 5 minutes and then air dried. Then the coplin jar was filled with diluted Giemsa stain and the blood film was placed into it for 30 minutes. The film was then washed with glass distilled water for 30 seconds

and dried in the air. A total of 100 cells were counted with oil immersion objective using Battlement method of counting for differential leukocyte count.

3.8 Packed Cell Volume (PCV)

The PCV was estimated by the method described by Schalm *et al.* (1975) as follow :

The sodium citrate treated venous blood of the experimental animal was filled in Wintrobe hematocrit tube with the help of Pasteur pipette. The free open end of the pipette was introduced to the bottom of the Wintrobe tube. The blood was expelled carefully and the pipette was withdrawn slowly keeping the open end always below the surface of the blood. In this way the tube was filled upto '0' mark on the left hand scale.

The tube was centrifuged at 6,000 rpm for 20 minute and PCV was read. The centrifugation of the tube was repeated for 20 minute twice or thrice. When the cell finally packed the percentage of PCV was recorded.

3.9 Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin concentration (MCHC)

The MCV, MCH and MCHC was calculated by the formula described by Swenson and Reece (1996) as follows :

$$\text{MCV in } \mu^3 = \frac{\text{PCV} \times 10}{\text{Number of erythrocytes (in million / mm}^3\text{ of blood)}}$$

$$\text{MCH in pg} = \frac{\text{Hb (g\%)} \times 10}{\text{Number of erythrocytes (in million / mm}^3\text{ of blood)}}$$

$$\text{MCHC in \%} = \frac{\text{Hb(g\%)} \times 100}{\text{PCV}}$$

3.10 Erythrocyte Sedimentation Rate (ESR)

The ESR was estimated by the method as described by Kolmer *et al.* (1969) as follows :-

The sodium citrate treated venous blood was mixed properly by inverting the tube three times. The Westergreen tube was filled exactly to the '0' mark. The bottom of the tube was pressed against the rubber stopper in the base of the rack and the finger was removed from the top of the tube. The tube was kept in an exactly vertical position by holding the tube firmly by the clip at the top of the rack. Reading was taken at 1st hour and 2nd hours interval.

$$\text{ESR (mm / hour)} = \frac{2 \times \text{Reading after 1}^{st}\text{ hour} + \text{Reading after 2}^{nd}\text{ hour}}{4}$$

3.11 Estimation of Serum Protein :

Total Serum Protein, albumin and globulin were estimated by biuret method as described by Murtuza (1998).

Principle :

Proteins bind with copper ions in an alkaline medium of the biuret reagent and produce a purple coloured complex, whose absorbance is proportional to the protein concentration.

Reagents Required

(1) Biuret Reagent

(2) Sodium Sulphite (Na_2SO_3) 28%

(3) Sodium Chloride (NaCI) 0.9%

(4) Protein Standard (2 mg/ml)

The Biuret reagent was stored at room temperature whereas protein standard was stored in refrigerator.

Procedure of total serum protein and serum albumin estimation :

0.2 ml serum was taken in a dry clean test tube. 5.8 ml sodium Sulphite was added in this test tube. The contents of this test tube was mixed well and left stand for 5 minutes. Thereafter, filtered the contents through Whatman Nubmer 44, dry filter paper and this filtrate was used for the estimation of albumin.

Four clean dry test tubes were labeled "Blank (B)" "Standard (S)", "Total Protein (T.P.)" and "Albumin (A)". 3 ml distilled water was taken in Blank test tube. 3 ml standard protein was taken in standard test tube. 0.1 ml serum was taken in test tube for estimation of total protein and mixed 2.9 ml 0.9% sodium chloride. For estimation of albumin, 3 ml above filtrate was taken in a test tube. 3 ml biuret reagent was mixed to each four test tubes. The contents were mixed well and kept in water at 37^0C for 10 minutes. Measured absorbance of standard (S), Total Protein (T.P.) and albumin (A) against Blank (B) on a photocolorimeter using green

filter at 540 nm wavelength. The difference between total protein and albumin was the value of globulin.

Calculations :

$$\text{Total Serum Protein in g/dl} = \frac{\text{O.D. of Total Protein}}{\text{O.D. of Standard}} \times 6$$

$$\text{Total Albumin in g/dl} = \frac{\text{O.D. of albumin}}{\text{O.D. of Standard}} \times 6$$

Serum Globulin in g/dl = Total Serum Protein – Serum albumin.

3.12 Estimation of Serum Uric Acid

Estimation of uric acid was carried out by standard method (Murtuza 1998).

Principle :

Uric acid reduces the colourless phosphotungstic acid to blue coloured phosphotungstus acid (Tungsten blue) in the presence of Sodium Carbonate.

Reagents :

1. Sodium tungstate, 10% : Dissolved 10 g of Na_2WO_4, $2H_2O$ in 100 ml Distilled water.
2. Sulphuric acid 2/3 N solution.
3. Tungstic acid : Added 50 ml 10% sodium tungstate, 50 ml 2/3 N Sulphuric acid and a drop of phosphoric acid with mixing to 800 ml water. Discarded when cloudy. Kept in brown bottle.

4. Phosphotungstic acid : to prepare a stock solution dissolved 50 g of sodium tungstate ($Na_2 WO_4$, 2 H_2O molybdate free) in about 400 ml of water. Added 40 ml of 85% phosphoric acid and refluxed gently for two hours. Cooled, transferred to a 500 ml flask and made the mark with water. Kept this in brown bottle. Diluted 1 to 10 for use and kept in brown bottle.

5. Sodium carbonate 10% : Dissolved 10 g anhydrous sodium carbonate in 100 ml. Distilled water. Kept in a polythene bottle.

6. Standard solution of uric acid (100 mg/100 ml):

   (a) Stock standard uric acid solution : Weighed out 100 mg of uric acid in a small beaker. Dissolved 60 mg of lithium carbonate in 15 to 20 ml of water in a test tube. Heated the solution to about 60^0C and poured on to the uric acid. Stirred until dissolved. When dissolved, transferred with washing to 100 ml of flask. Added 2 ml of 40% formalin and then slowly with shaking, added 1 ml of 50% v/v acetic acid. Made to the mark with water and mixed. Kept in a well stoppered bottle away from the light.

   (b) Standard solution for use : Diluted 1 ml of the stock standard to 200 ml.

Procedure.:

Took 0.6 ml serum in centrifuge tube, added 5.4 ml of dilute Tungstic acid with shaking and centrifuged it. Took three test tubes, labeled test, standard and blank. Added 3 ml of the supernatant, 3 ml of the dilute standard and 3 ml of water in corresponding tubes. Added 0.6 ml of the sodium carbonate solution to each and followed with 0.6 ml of dilute phosphotungistic acid. Mixed and placed in a 25^{0}C water bath for 30 minutes. Read at 700 millimicrons using a red filter.

Calculation :

mg uric acid per 100 ml serum

$$= \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 0.015 \times \frac{100}{0.3} = \frac{\text{R.U.}}{\text{R.S.}} \times 5.0$$

3.13 Determination of Serum Creatinine

The serum creatinine was estimated by spectrophotometer following the procedures as described by Murtuza (1998).

Reagent :

1. Sodium tungstate 5% solution : Dissolved 5 gm sodium tungstate in water and made the volume to 100 ml.
2. Sulphuric acid 2/3 N solution.
3. Stock standard of creatinine solution : Dissolved 1 g of dry creatinine in 0.1 N hydrochloric acid and made the volume 1 litre with the acid. It contained 1 mg creatinine per ml.

4. Standard creatinine solution (0.04 mg/ml) : Diluted 4 ml stock standard to 100 ml.
5. Picric acid, 0.04 M solution (9.16 g/1000 ml) : Picric aid was taken on a pad of filter paper. 9.16 g of dry picric acid was dissolved in water and was made upto one litre.
6. Sodium hydroxide, 0.75 N solution.

Procedure :

Took 2 ml of serum in a test tube, added 2 ml of distilled water; added 2 ml of 5% sodium tungstate and mixed. Added 2 ml of 2/3 N sulphuric acid, drop by drop with constant shaking. Allowed to stand for ten minutes and filtered.

Took 3 ml of filtrate (= 0.75 ml serum) in a test tube, added 1 ml of the picric acid and 1 ml of sodium hydroxide. Allowed to stand for 15 minutes. Prepared reagent blank, substituting water for the serum and for the standard 2 ml of standard and 2 ml of water. Added 2 ml 5% sodium tungstate and 2 ml of 2/3 N Sulphuric acid and proceeded as for the test. Reading was taken at 520 nm.

Calculation :

$$\text{Creatinine (mg per 100 ml serum)} = \frac{\text{O.D. of unknown}}{\text{O.D. of standard}} \times 0.030 \times \frac{100}{0.75}$$

$$= \frac{\text{O.D. of unknown}}{\text{O.D. of standard}} \times 4.0$$

3.14 Determination of Serum Transaminases

Serum Transaminases were estimated by the method as described by Murtuza (1998). Following reagents were prepared :

1. Buffer :

   (a) 11.876 gm of $Na_2 HPO_4$, $2H_2O$ was dissolved in distilled water and was made upto 1000 ml.

   (b) 9.078 gm of $KH_2 PO_4$ was dissolved and made upto 1000 ml with distilled water. Phosphate Buffer (pH 7.45) was made by mixing 825 ml of (a) and 175 ml of (b).

2. Substrate for SGOT : 2.66 g of dl aspartic acid and 30 mg α oxologlutaric acid was dissolved in 20.5 ml N NaOH by gentle heating. This volume was made upto 100 ml with phosphate buffer solution.

3. Substrate for SGPT :1.7 g of dl-alanine and 30 mg α oxologlutaric acid was dissolved in 20 ml of phosphate buffer to which 0.5 mlof N NaOH was added. This solution was diluted to 100 ml with buffer phosphate. 4 drops of chloroform was added ad stored in refrigerator.

4. Aniline citrate reagent : 50 g of citric acid was dissolved in 50 ml of distilled water. Equal parts of citric acid solution and redistilled aniline were taken and mixed. This reagent was kept in brown bottle for further use.

5. Colour reagent : It was prepared by dissolving 200 mg 2, 4-dinitrophenyl hydrazine in hot N HCI and diluted to 1000 ml with the same HCI. It was also kept in dark in brown bottle.
6. Sodium Hydroxide : 0.4 N solution was prepared by dissolving 4 parts of N NaOH with 6 parts of distilled water.
7. Pyruvate standard : 22 mg of sodium Pyruvate was dissolved in 100 ml of buffer solution and was kept for few days in refrigerator.

Principle :

Pyruvate and oxaloacetate so formed are treated with 2,4 DNPH, the brown colour produced is measured at 520 millimicrons.

Procedure :

The separate standard calibration curve of SGOT and SGPT were prepared.

For test serum separate test tubes were taken for SGOT, SGPT and their respective Blank and were labeled accordingly. 1 ml of respective substrate (SGOT and Blank and SGPT and Blank) was taken in the tube and was incubated for 5 minutes at 37^0C in water bath. 0.02 ml of serum was added to the 'Test' test tubes and were mixed and incubated at 37^0C for 1 hour (in case of SGOT) or 30 minutes (in case of SGPT). After incubation 0.05 ml of aniline citrate reagent was added in each tube. 0.2ml of serum was added to the

blank and was left for 5 minutes. 1ml of 2,4 dinitro phenylhydrazine was added into each tube and left in the water bath for another 15 minutes. 10 ml of 0.4 N sodium hydroxide were added in each tube and were mixed by inversion. It was further allowed to stand for 15 minutes in dark for colour development. O.D. was taken in photoelectric coorimeter at 520 nm against respective blank for SGOT and SGPT.

The values of SGOT or SGPT (in mIu per ml) were taken directly from their respective standard calibration curve by placing the O.D. of test serum.

3.15 Determination of Serum Alkaline Phosphatase (SALP)

Serum alkaline phosphatase was estimated by the method described by Murtuza (1998). Following reagents were prepared as described

1. Phenyl phosphate solution (0.01 M).
2. Buffer solution (0.1 M) : 3.18 g of anhydrous sodium carbonate and 1.68 g of sodium bicarbonate was dissolved in distilled water and was made upto 500 ml.
3. Buffer Substrate (PH 10): Equal volume of phenyl phosphate and buffer solution was mixed to prepare buffer substrate. It was prepared at the time of use.
4. Sodium Hydroxide (0.5 M) : Dissolved 10 g of sodium hydroxide in distilled water and was made upto 500 ml.

5. Sodium Bicarbonate (0.5 M) : Dissolved 21 gm of sodium bicarbonate in distilled water and was made upto 500 ml.

6. 4-Aminophenazone (4-aminoantipyrine) : 3 gm of 4-aminophenazone was dissolved in distilled water and was made upto 500 ml.

7. Potassium ferricyanide : 12 gm of potassium ferricyanide was dissolved in distilled water and was made upto 500 ml.

8. Stock standard phenol solution : 0.5 gm of pure crystalline phenol was dissolved in N/10 hydrochloric acid and was made up to 500 ml with N/10 HCI.

9. Working standard phenol solution : 1 ml of stock standard phenol solution was dissolved upto 100 ml with distilled water (0.01 mg/ ml).

Principle :

Phenol is liberated by the action of serum alkaline phosphatase on phenyl phosphate. Phenol reacts with 4 – aminophenazone in the presence of oxidizing agent to form red purple compound, which is estimated colorimetricaly.

Procedure :

Four test tubes were taken for one test and were labeled as T (test), C (control), S (Standard) and B (Blank). 2 ml of buffered substrate was taken in T and C and was placed in water bath at 37^0C

for few minutes. 0.1 ml of serum was added to T and was incubated exactly for 15 minutes. 1.1 ml of buffer solution was taken in S and B. 1 ml of working standard phenol solution was added in S and 1 ml of distilled water in B. These two tubes were kept at room temperature for some time. 0.8 ml of sodium hydroxide and 1.2 ml of sodium bicarbonate were added in all the four tubes. 0.1 ml of serum was added in C. 1 ml 4 aminophenazone was added to each tube and mixed. Now 1 ml of potassium ferricyanide was mixed in all the tubes. Optical densities of all the solution were taken against blank (B) at 520 nm. Serum alkaline phosphatase activity was calculated as under:

$$\text{SALP (in KA unit)} = \frac{\text{O.D. of 'T'} - \text{O.D. of "C"}}{\text{O.D. of 'S'}} \times 10$$

3.16 Estimation of Sodium and Potassium

The estimation of sodium and potassium was carried out by Systronics flame photometer Burner unit 121 as described by Murtuza (1998):-

Reagents :

Sodium standards - For calibration cure the standard solution ranging from 100 to 180 milli equivalents of sodium per litre at a dilution of 1 : 100 was prepared.

Stock Standard : 5.85 g of NaCI was dissolved in glass distilled water and was diluted to 1 litre. It was containing 100 milli-equivalents of sodium per litre.

Working standards : Pipetted 10, 11, 12, 13, 14, 15, 16, 17 and 18 ml stock standard into 1 litre volumetric flasks and diluted to the mark with glass distilled water. These working standards were equivalent to 100, 110, 120, 130, 140, 150, 160, 170 and 180 milli – equivalents of sodium per litre at 1 : 100 dilution. Took the reading of these standards in the flamephotometer, plotted the readings against equivalent sodium content in milli equivalents per litre and prepared a calibration curve.

Potassium standards : For calibration, prepared the standard solution ranges from 3 to 7 milli equivalents of potassium per litre, at 1 : 100 dilution.

Stock standard : Dissolved 0.746 g of KCl in water and diluted to 1 litre with glass distilled water. It contained 10 milli equivalents of potassium per litre.

Working standard : Pipetted 3, 4, 5, 6 and 7 ml of stock standard into 1 litre volumetric flasks and diluted to the mark with glass distilled water. These working standards were containing 3,4,5,6 and 7 milli equivalents potassium per litre, at a dilution of 1 : 100. Prepared calibration curve from the above working standards.

A single working standard was used for the determination of serum sodium and potassium. It was prepared by diluting 18 ml of sodium stock standard and 7 ml of potassium representing 180 milli equivalent of sodium and 7 milli equivalent of potassium per litre at a dilution of 1 : 100.

Procedure : The serum dilution of 1 : 100 (0.1 ml serum diluted to 10 ml with glass distilled water) was used for sodium and potassium. Standard solutions were used along with the unknown samples. The samples was analyzed according to the directions given in the booklet which accompany the instrument. The sodium (or potassium) concentration of the sample was obtained by the calculation given below.

Flame photometer was setup properly; connected with Indane gas cylinder and with air compressor. The filter (Sodium or Potassium) was put in place. The instrument was switched on and the air pressure was adjusted to about 0.4 kg/cm. The knob of the gas cylinder was turned and the burner of the flame photometer was lighted. The flame was adjusted to non-luminous. First of all glass distilled water was passed through suction tube to spray over the flame. The galvanometer reading was made to zero when distilled water was sprayed over flame by proper adjuster. The distilled water was removed and the suction tube was placed into standard solution. Adjusted the galvanometer reading to 100 with adjuster. Took out the standard solution and put again distilled water and again adjusted the galvanometer reading to zero. Again checked with standard solution and saw that the reading was 100. The instrument was now ready for determining the unknown sample. The diluted serum sample was now sucked by suction tube. The reading of galvanometer was noted.

After taking the reading of about five samples, checked zero reading with distilled water and 100 by standard solution. When required readjusted the reading again.

After taking the reading of samples, first the Indane gas was disconnected and then galvanometer and air compressor was switched off.

Calculation for sodium :

$$\text{Value of Sodium} = \frac{180\ \text{mEq/L}}{100} \times T = 1.8 \times T\ \text{mEq/L}$$

Where, T = reading of unknown sample of sodium.

Calculation for Potassium :

$$\text{Value of Potassium} = \frac{7\ \text{mEq/L}}{100} \times X = 0.07\ X\ \text{mEq/L}$$

Where, X = reading of unknown sample of potassium.

Statistical Analysis :

Data were analysed as described by Snedecor and Cochran (1976).

□□□□□

Table-1

ANALYSIS OF VARIANCE OF DIFFERENT PARAMETERS

Parameters	Sources of variation	Degree of Freedom	C.S.S.	Mean squares (M.S.)	F. Value
Total Erythrocyte count	Between group	4	31.58	7.89	5.06*
	Error	25	39.02	1.56	
Hemoglobin concentration	Between group	4	40.60	10.15	2.74^{NS}
	Error	25	92.62	3.70	
Packed cell volume	Between group	4	373.33	93.33	2.63^{NS}
	Error	25	884.05	35.36	
Mean corpuscular volume	Between group	4	389.82	97.45	0.744^{NS}
	Error	25	3267.44	130.69	
Mean corpuscular Hemoglobin	Between group	4	64.17	16.03	0.97^{NS}
	Error	25	412.05	16.48	
Mean corpuscular Hemoglobin concentration	Between group	4	93.21	23.30	1.24^{NS}
	Error	25	470.21	18.81	
Blood clotting time	Between group	4	13.53	3.38	2.19^{NS}
	Error	25	38.58	1.54	
Erythrocyte sedimentation rate	Between group	4	0.53	0.13	1.88^{NS}
	Error	25	1.73	0.06	
Total leucocyte count	Between group	4	28.08	7.02	1.62^{NS}
	Error	25	107.83	4.31	
Lymphocytes	Between group	4	353.11	88.27	6.35*
	Error	25	389.86	15.59	
Monocytes	Between group	4	2.51	0.62	1.06^{NS}
	Error	25	14.86	0.59	
Neutrophils	Between group	4	292.84	73.21	4.12**
	Error	25	444.52	17.78	

Parameters	Sources of variation	Degree of Freedom	C.S.S.	Mean squares (M.S.)	F. Value
Eosinophils	Between group	4	3.11	0.77	0.69^{NS}
	Error	25	27.86	1.11	
SGOT	Between group	4	1067.14	266.78	1.86^{NS}
	Error	25	3591.16	143.65	
SGPT	Between group	4	284.81	71.20	1.38^{NS}
	Error	25	1286.66	51.47	
Alkaline Phosphatase	Between group	4	1696.97	424.24	5.26*
	Error	25	2015.39	80.616	
Total serum Protein	Between group	4	101.89	25.47	62.14*
	Error	25	10.24	0.40	
Albumin	Between group	4	19.21	4.80	60.00*
	Error	25	2.14	0.08	
Globulin	Between group	4	32.79	8.19	36.59*
	Error	25	5.61	0.22	
Serum uric acid	Between group	4	1.60	0.40	1.86^{NS}
	Error	25	5.35	0.21	
Serum creatinine	Between group	4	0.65	0.16	0.26^{NS}
	Error	25	15.41	0.61	
Serum sodium	Between group	4	412.58	103.14	1.47^{NS}
	Error	25	1753.44	70.13	
Serum potassium	Between group	4	4.39	1.097	1.28^{NS}
	Error	25	21.39	0.85	

* Significant at 1% level

** Significant at 5% level

NS – non significant

Chapter - 4

Results and Discussion

RESULTS AND DISCUSSION

4.1 Hemograms of the Experimental Animals

4.1.1 Total Erythrocyte Count (TEC), Hemoglobin (Hb) Concentration and Packed Cell Volume (PCV)

The value (Mean ± SE) of TEC, Hb and PCV in circulating blood of noncycling heifers aging 10-13 months, 18-21 months and 27-30 months, cycling heifers and lactating cycling cows have been presented in table-2, The values of TEC, Hb and PCV were respectively 7.06 ± 0.47 million/ mm^3, 12.03 ± 0.80 g/dl and 32.33 ± 2.40% in 10-13 months heifers ; 6.43±0.76 million/mm^3, 11.92 ± 0.78 g/dl and 33.83 ± 2.39% in 18-21 months heifers; 8.20 ± 0.53 million / mm^3, 14.66 ± 0.54 g/dl and 39.33 ± 1.08 % in 27-30 months heifers ; 8.35 ± 0.39 million / mm^3, 13.33 ± 0.98 g/dl and 36.67 ± 2.11% in cycling heifers and 5.67 ± 0.23 million / mm^3, 11.50 ± 0.75 g/dl and 29.08 ± 3.52% in cycling lactating cows.

Individually the TEC ranged from 5.26 million/ mm^3 to 8.38 million/mm^3, 4.89 million/mm^3 to 10.03 million/mm^3, 6.19 million/mm^3 to 10.15 million/mm^3, 7.60 million/mm^3 to 10.29 million/mm^3 and 5.03 million/mm^3 to 6.56 million/mm^3 ; Hb concentration ranged from 10.2 g/dl to 15.5 g/dl, 10 g/dl to 15 g/dl, 13 g/dl to 16 g/dl, 10 g/dl to 17 g/dl and 10 g/dl to 15 g/dl and PCV ranged

from 25% to 42%, 30% to 45%, 35% to 42%, 30% to 45% and 22.5% to 45% respectively, in 10-13 months, 18-21 months, 27-30 months non-cycling heifers, cycling heifers and cycling lactating cows.

The mean TEC value of lactating cows was significantly ($P<0.01$) lower than the TEC value recorded in noncycling heifers of 27 to 30 months and cycling heifers. However, the value was similar ($P>0.05$) to the TEC values estimated in noncycling heifers of 10 to 13 months and 18 to 21 months. Among the non cycling group of heifer the TEC value of 27 to 30 months was similar to the TEC value of 10 to 13 months heifers, while it was significantly ($P<0.05$) higher than the TEC value estimated in 18 to 21 months heifers.

The Hb concentration in lactating cows was significantly ($P<0.01$) lower than the Hb concentration of 27-30 months non-cycling heifers. This value was similar ($P>0.05$) to the Hb level recorded in cycling heifers and noncycling heifers of 10-13 months and 18-21 months. The Hb level of 27-30 months heifers was higher ($P<0.05$) than the Hb level of 10-13 months and 18-21 months noncycling heifers and it was similar ($P>0.05$) to the Hb level of cycling heifers.

The PCV value obtained in lactating cows was significantly ($P<0.01$) lower than the PCV value recorded in 27-30 months noncycling heifers and it was similar ($P>0.05$) to the PCV value recorded in 10-13 months and 18-21 months noncycling heifers.

Table-2

Total Erythrocyte Count (TEC), Hemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Blood Clotting Time (BCT) and Erythrocyte Sedimentation Rate (ESR) in different group of Animals (Mean ± SE)

Animal Group		TEC ($\times 10^6/mm^3$)	Hb (g/dl)	PCV (%)	MCV (μ^3)	MCH (pg)	MCHC (%)	BCT (Mint.)	ESR (mm/hr)
Non Cycling Group	10-13 Months	$7.06^{ab} \pm 0.47$	$12.03^{ab} \pm 0.80$	$32.33^{ab} \pm 2.40$	$47.21^{a} \pm 5.21$	$17.53^{a} \pm 1.81$	$37.39^{ab} \pm 0.95$	$6.50^{a} \pm 0.43$	$0.29^{a} \pm 0.04$
	18-21 Months	$6.43^{ab} \pm 0.76$	$11.92^{a} \pm 0.78$	$33.83^{ab} \pm 2.39$	$55.24^{a} \pm 6.03$	$19.64^{a} \pm 2.37$	$35.31^{a} \pm 0.93$	$8.00^{b} \pm 0.63$	$0.46^{a} \pm 0.12$
	27-30 Months	$8.20^{a} \pm 0.53$	$14.66^{b} \pm 0.54$	$39.33^{b} \pm 1.08$	$49.33^{a} \pm 4.39$	$18.31^{a} \pm 1.60$	$37.42^{ab} \pm 1.82$	$6.66^{ab} \pm 0.61$	$0.48^{a} \pm 0.11$
Cycling group	Heifers	$8.35^{a} \pm 0.39$	$13.33^{ab} \pm 0.98$	$36.67^{b} \pm 2.11$	$44.43^{a} \pm 3.33$	$16.13^{a} \pm 1.40$	$36.58^{ab} \pm 2.64$	$6.50^{a} \pm 0.41$	$0.37^{a} \pm 0.12$
	Lactating cows (1^{st} to 4^{th} lactation	$5.67^{b} \pm 0.23$	$11.50^{a} \pm 0.75$	$29.08^{a} \pm 3.52$	$50.57^{a} \pm 3.87$	$20.20^{a} \pm 0.53$	$40.65^{b} \pm 1.91$	$6.00^{a} \pm 0.38$	$0.58^{a} \pm 0.12$

The PCV value of cycling heifer was significantly ($P<0.05$) higher than the PCV value recorded in lactating cows while it was similar ($P>0.05$) to the PCV value of in noncycling heifers of 10-13 months, 18-21 months and 27-30 months.

The total Erythrocyte count in circulation of crossbred noncycling heifers, cycling heifers and cycling lactating cows of present investigation falls within the range of TEC reported by Schalm et al. (1975) in cows. The TEC value of lactating cows estimated during present study was lower than the total number of erythrocyte recorded in adult cows born and maintained in temperate climate (Holman, 1956; Schalm *et al.*, 1975). The lower value of TEC estimated in lactating cows was also associated with lower value of Hb and PCV in this group (Table-2).

The TEC and PCV reported by Smith (1959) is more than the TEC and PCV value of the present experiment which might be due to the fact that the blood samples of Smith (1959) included cows which were pregnant, lactating and dry. The TEC value of lactating cows was less than the heifer of all age groups. A similar pattern of higher TEC is reported (Shukla *et al.* 1982) in Marwari sheep where TEC was lower in lactating ewes than in dry ewes. The decline in the red cell number might be related to milk yield (Hewett, 1974) as high producers tend to develop anemia more frequently (Whitlock et al, 1974). In general non lactating cows have higher RBC, Hb and PCV

value than lactating cows (Noonan, 1978). The implication of climate factor may also be considered for slight lower TEC in our crossbred animals.

The concentration of hemoglobin depends upon different factors like breed, age, exercise, rest, excitement, ambient temperature, agroclimatic conditions, feeding and management practices used (Safi et al 1987, Swenson and Reece 1996). Some of the above factors might be responsible for variation of Hb at various ages of cattle in our experiment. The RBC count and Hb concentration of animal of present study is lower than the values reported by Mithuji et al (1966) in Kankarej cattle which may be due to the fact that they had selected the cows randomly without any consideration about the age and lactation. Mishra and Biswal (1961) also reported more RBC count, Hb concentration and PCV value in Orissa cattle than the value estimated during present experiment. However, they did not consider the sex, age and lactation during selecting the animals. The explanation for the lower value of TEC and Hb concentration in the adult lactating cows in present investigation lies with the available evidence in respect of fluctuation of Hb and TEC with age and lactation (Mishra and Biswal 1961, Shukla et al., 1982; Khadjeh and Papahn, 2002). From the experiment a relation between TEC and Hb concentration can be made because the Hb concentration is directly related to RBC concentration in physiologically normal mammals

having optimum functioning of hematopoietic system (Swenson and Reece, 1996). The value of Hb concentration increases with the increase in the number of erythrocytes (table –2) in our estimations which gets support from the report of Kumar et al (1990) where increasing trend of Hb concentration was also found with increase in the number of erythrocytes in Murrah heifers. In present study no any particular trend in respect of increase or decrease in Hb concentration as per age of crossbred heifers was found which was also reported by some others investigators (Kumar *et al.* 1990; Sharma *et al* 1985; Mishra and Biswal 1961, Holman 1956) where no any particular trend was set up with advancing age. We estimated the lesser number of RBC in cows than that of heifers (table-2) which is also in agreement with the findings of Deshpande *et al.* (1987) who reported lesser number of RBC in cows than in heifers. However, the TEC detected during the present study is within normal physiological range reported by Schalm *et al.* (1975) and Holman (1956).

In the present study almost same trend was established with Hb and PCV (table-2). Where the Hb concentration is more the PCV level is also high and the group having lower level of Hb has lower level of PCV also. The increasing PCV value in noncycling heifers in present case is in agreement with the findings recorded by Schalm *et al.* (1975) where the PCV has increasing trend in the similar age group of 11-12 months, 15-19 months and 20-36 months

in Jersey female cattle. In the present investigation Hb, PCV and TEC were found less in lactating cows than the other groups which gets support from the report of Hewett (1974) and Rowlands *et al.* (1977). However, the value of TEC, Hb concentration and PCV of 10-13 months noncycling heifers are similar with the values estimated earlier in the same cattle farm in the 10-11 months of crossbred animals. (Prabha et al., 1999).

4.1.2 Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC)

The value for MCV, MCH and MCHC for different groups of animals are presented in table-2. The value (Mean ± SE) for MCV were 47.21 ± 5.21 μ^3, 55.24 ± 6.0.3 μ^3, 49.33 ± 4.39 μ^3, 44.43 ± 3.33 μ^3, and 50.57 ± 3.87 μ^3, in 10-13 months, 18-21 months, 27-30 months non cycling heifers, cycling heifers and cycling lactating cows respectively. The MCH values (Mean ± SE) were 17.53 ± 1.81 pg, 19.64 ± 2.37 pg, 18.31 ± 1.60 pg, 16.13 ± 1.40 pg and 20.20 ± 0.53 pg in the respective group of animals. The values (Mean ± SE) for MCHC were 37.39 ± 0.95%, 35.31 ± 0.93 %, 37.42 ± 1.82 %, 36.58 ± 2.64% and 40.65 ± 1.91% respectively.

The values of MCV, MCH and MCHC in individual group of animals ranged from 29.83 μ^3 to 64.60 μ^3, 12.53 pg to 23.95 pg and 35.48 % to 42.00 % in 10-13 months heifer ; 29.91 μ^3 to 69.03 μ^3, 9.97

pg to 26.62 pg and 33.33% to 38.57 % in 18-21 months heifers; 34.48 μ^3 to 67.85 μ^3, 14.78 pg to 25.84 pg and 32.5% to 45.7% in 27-30 months heifers ; 34.01 μ^3, 54.81 μ^3, 12.50 pg to 21.49 pg and 30.00% to 48.57% in cycling heifers and 44.72 μ^3, to 68.59 μ^3, 19.51 pg to 22.86 pg and 33.33% to 44.44 % in cycling lactating animals respectively.

The MCV and MCH values between different groups of animals were similar ($P>0.05$). The MCHC value recorded in lactating cows was significantly ($P<0.05$) higher than the MCHC value of the 18-21 months noncycling heifers and it was similar ($P>0.05$) to the value of MCHC recorded in noncycling heifers of 10-13 months, 27-30 months and cycling heifers.

The values of MCV, MCH and MCHC indicates the health status of animals in respect of erythropoiesis and certain mineral metabolism. The MCV provides the average cell size in μ^3, MCH expresses the average weight of hemoglobin in the erythrocyte while MCHC gives the average percentage of MCV which the hemoglobin occupies.

By over viewing the values (Mean ± SE) and the range among the individual animals of each group reveals that though the values between different age group are not identical and fluctuated within the narrow range and hence the difference in mean value between the groups of the animals during the present studies are not

significant. However, uniformly a little higher MCV in individual animals of 18-21 months than any groups was recorded. The increasing age of the heifers has no any relation with the MCV value. Moreover, the higher value of MCV in 18-21 months heifers suggest that certain intrinsic mechanisms responsible for erythropoisis specially in respect of size of RBC is effective at age group of 18-21 months of noncycling heifers leading to a little higher MCV value in these group of heifers than any other groups. This suggests that certain age of growing period of the crossbred animals in tropical climate also affects the erythropoitic system as reported earlier in case of the animals adapted in tropical climate (Swenson and Reece, 1996). On comparing MCV and MCH of different groups it is found that with increase in MCV value the MCH also increases but on viewing the TEC of different groups it is found that there is existence of reciprocal fluctuation between size and number of erythrocytes and almost the same trend has also been reported by some other investigators in different age groups of animals (Prabha *et al.* 1999; Kumar *et al.* 1990; Sharma *et al.* 1985; Schalm *et al.* 1975). It means that there may be the existence of some intrinsic compensatory mechanism due to which the lower number of erythrocytes may be compensated by increase in the size of the cells. (Schalm *et al.* 1975).

The value of MCV of 10-13 months and 18-21 months heifers are more than the MCV reported by Schalm *et al.* (1975) in

11-12 months and 15-19 months female cattle and due to the above discussed compensatory mechanism the RBC count in our group is less than that of the respective group of Schalm *et al.* (1975). However the MCV of 27-30 months heifers of our study is similar to the MCV of 20-36 months female cattle and in the same way RBC count is also similar (Schalm *et al.* 1975).

The value of MCV, MCH and MCHC in the 10-13 months non cycling heifers are similar to the values reported by Prabha et al (1999) in the same cattle farm in the age group of 10-11 months.

The MCV and MCH in 27-30 months heifers are more than that of 10-13 months heifers and less than 18-21 months. The same trend was also found by Kumar et al (1990) in Murrah buffaloes who reported the MCV and MCH in 25-30 months of heifer more than that of 12 months heifer but less than 20-24 months heifers. Higher value of 10-13 months, 18-21 months and 27-30 months heifers are similar to the MCHC found in 12 months, 20-24 months and 25-30 months Murrah heifers (Kumar *et al.* 1990).

In our findings the MCV and MCH of lactating cows are lower than that of Red Khandhari cows (Deshpande *et al.*, 1987) Which may be due to the difference in Hb, PCV and number of erythrocytes.

Hemoglobin concentration of present experimental cows is similar to the Hb concentration of crossbred cows and the mean corpuscular volume is less than that so the portion of erythrocytes

occupied by hemoglobin will be obviously higher and that's why MCHC of experimental cows are higher than those crossbred postpartum cows (Mehere et al 2002).

4.1.3 Blood Clotting Time (BCT)

The blood clotting time recorded in different groups of crossbred cattle are presented in table 2. The BCT (Mean ± SE) recorded in noncycling heifers of 10-13 months, 18-21 months, 27-30 months; cycling heifers and cycling lactating cows were 6.50 ± 0.43 minute, 8.00 ± 0.63 minute, 6.66 ± 0.61 minute, 6.50 ± 0.41 minute and 6.00 ± 0.38 minute respectively. Individually the BCT ranged between 5 minute to 7 minute in 10-13 months heifers, 6 minute to 9 minute in 15-18 months heifers, 5 minute to 7.5 minute in 27-30 months heifers, 5.25 minute to 7.25 minute in cycling heifer and 5 minute to 7.5 minute in cycling lactating cows. The BCT of 18-21 months heifers is higher ($P<0.05$) than the BCT of 10-13 months heifers, cycling heifers and cycling lactating cows. The other values of BCT are statistically similar ($P>0.05$) to each other.

The BCT recorded in 10-13 months heifers is similar to the BCT in 10 months calves (Greatorex 1954). The values of BCT recorded in our experiment is similar to the value reported by Osbaldiston *et al.* (1970) in bovines.

The BCT in different age groups of crossbred animals falls within the range (3-15 minutes) reported by Coles (1974) in bovines and is also supported by Mishra and Biswal (1961) in Orissa cattle.

4.1.4. Erythrocyte Sedimentation Rate (ESR)

The erythrocyte sedimentation rate recorded in different group of animals are presented in table 2. The ESR in non cycling heifers of 10-13 months, 18-21 months, 27-30 months; cycling heifers and cycling lactating cows was 0.29 ± 0.04 mm/hr., 0.46 ± 0.12 mm/hr, 0.48 ± 0.11 mm/hr, 0.37 ± 0.12 mm/hr and 0.58 ± 0.12 mm/hr respectively. The ESR recorded in different groups of animals are statistically similar ($P>0.05$) to each other.

The ESR recorded in this experiment agree with the view that among the domestic animals the erythrocyte of ruminatns show little or no natural tendency to form rouleax. Therefore, there is little or no ESR in healthy condition and only a serious tissue alteration in disease results in significant rouleax formation (Schalm *et al.*, 1976).

ha and Singh ( ) also found very less ESR in the same animal herd. They observed the sedimentation of RBC for 24 hours to get some impressive value, and it was 8.16 ± 0.16 to 18.30 ± 1.83 mm/24hr. In the same way Ferguson (1937), too, observed the settling of RBC for 7 hr. and got it only 2.4 mm in 7 hours.

In the present experiment it has been observed that age and cyclicity has no influence on ESR as there was no any significant difference observed in the ESR of different groups.

Our finding support the view made by Schalm et al (1975) that cow has the minimal ESR. The mean value of ESR in crossbred bovine (Hariana × HF) ranged between 0.29 to 0.58 mm/hr. while the ESR of Dog, Cat, Horse and Pig are reported to be 6-10 mm/hr, 7-27 mm/hr, 15-38 mm/20 mint and 1-14 mm / 60 mint respectively (Schalm *et al.* 1975).

The preset finding is approaching towards the fact stated by Swenson and Reece (1996) that increase in globulin content of plasma hastens the agglutination and settling of RBC. In present investigation blood serum globulin increases from Group I to group V (table 4) and same tendency is also observed with ESR except the group IV, although, the difference is non significant, but this may be due to the fact that ESR also depends upon plasma fibrinogen content (Schalm et al., 1975 ; Swenson and Reece, 1996) which is not estimated during the present experiment.

Any available information could not satisfy the exact physiology of ESR in different growing ages and reproductive status in female cattle and thus it needs some more detailed studies in such animals.

4.1.5 Total Leukocytic Count (TLC) and Differential Leukocytic Count (DLC)

The total leukocytic count and differential leukocytic count (Mean ±SE) of female crossbred cattle in different age group has been presented in table 3. The circulating TLC for noncycling heifer of 10-13 months, 18-21 months and 27-30 months, cycling heifers and cycling lactating cows were 8.82 ± 0.83 thousand / mm^3, 10.13±0.53 thousand / mm^3, 9.37 ± 1.15 thousnad / mm^3, 8.46 ± 0.98 thousand / mm^3 and 7.23 ± 0.57 thousand / mm^3 of blood respectively. The TLC in lactating cow was significantly ($P<0.05$) lower than the TLC value of 18-21 months noncycling heifers and it was similar ($P>0.05$) to the value of TLC recorded in noncycling heifers of 10-13 months, 27-30 months and cycling heifers. Some fluctuation was recorded in TLC value of different groups of animals. The circulating TLC ranged between 5.850 to 11.450 thousand / mm^3, 8.450 to 11.650 thousand/mm^3 4.750 to 13.300 thousand/mm^3, 4.400 to 10.950 thousand/mm^3 and 5.300 to 8.600 thousand/mm^3 of blood in 10-13 months, 18-21months, 27-30 months noncycling heifers, cycling heifers and cycling lactating cows respectively.

The record of different leukocytic count reveals that the percent of neutrophils was 26.5 ± 1.72% in 10-13 months heifers, 26.83 ± 1.74 % in 18-21 months heifers, 26.33 ± 1.40 % in 27-30

months heifers, 24.66 ± 2.38 % in cycling heifers and 18.50 ± 1.05% in cycling lactating cows.

Individually the percent of neutrophils fluctuated between 21% to 34% in 10-13 months heifers, 23% to 34% in 18-21 months heifers, 20% to 30% in 27-30 months heifers, 16% to 32% in cycling heifers and 15% to 21% in cycling lactating cows.

The neutrophil count in lactating cow was significantly lower than that of noncycling ($P<0.01$) and the cycling ($P<0.05$) heifers. The percent of neutrophil counted in 10-13 months heifers 18-21 months heifers, 27-30 months heifers and cycling heifers were similar ($P>0.05$) to each other.

The percent of lymphocytes in 10-13 months heifers was 70.00±1.73%, in 18-21 months heifers 68.16±1.62% , in 27-30 months heifers 69.16±1.35%, in cycling heifers 71.66±1.92% and in cycling lactating cows it was 77.83±1.35%. Individually the percentage of lymphocytes counted were varying from 62% to 74% in 10-13 months heifers, from 62% to 73% in 18-21 months heifers, from 65% to 74% in 27-30 months heifers, from 65% to 78% in cycling heifers and from 74% to 83% in cycling lactating cows. The lymphocyte percent of cycling lactating cow was significantly higher than the lymphocyte percent of noncycling heifers ($P<0.01$) and the cycling heifers

($P<0.05$). The percentage of lymphocytes counted in 10-13 months heifers, 18-21 months heifers, 27-30 months heifers and cycling heifers were similar ($P>0.05$) to each other.

The percent (Mean ± SE) of Eosinophil counted in the animal group of 10-13 months heifers, 18-21 months heifers, 27-30 months heifers, cycling heifers and cycling lactating cows was 2.50±0.34%, 3.16±0.40%, 2.83±0.46%, 2.33±0.49% and 2.33±0.49% respectively. The different values of Eosinophil count obtained during present study were similar ($P>0.05$) to each other. Individually a very narrow range of fluctuation in Eosinophil were recorded. The eosinophil count ranged between 2% to 4% in noncycling heifers of 10-13 months, 18-21 months and 27-30 months while in cycling heifers and lactating cow it ranged from 1% to 4%.

The percent of monocytes observed during present study was very low. Even some slides were not showing any monocytes during the count. On statistical analysis the values (Mean ± SE) of different groups were similar ($P>0.05$). The percent of monocytes observed in 10-13 months, 18-21 months and 27-30 months noncycling heifers, cycling heifers and cycling lactating cows were 1.00 ± 0.36%, 1.83±0.16%, 1.66±0.21%, 1.33±0.33% and 1.33±0.42% respectively.

The circulating leucocytic count vary considerably among the animals. This variation might be due to different physiological

Table – 3

Total and Differential Leukocytic Count (Mean ± SE) in different group of crossbred animals (Heifers and Cows)

	Animal Group	TLC (thousand/mm^3)	DLC (%)			
			Neutrophils	Lymphocytes	Eosinophils	Monocytes
Non Cycling Group	10-13 Months	$8.82^{ab} \pm 0.83$	$26.5^{a} \pm 1.72$	$70.00^{a} \pm 1.73$	$2.50^{a} \pm 0.34$	$1.00^{a} \pm 0.36$
	18-21 Months	$10.13^{a} \pm 0.53$	$26.83^{a} \pm 1.74$	$68.16^{a} \pm 1.62$	$3.16^{a} \pm 0.40$	$1.83^{a} \pm 0.16$
	27-30 Months	$9.37^{ab} \pm 1.15$	$26.33^{a} \pm 1.40$	$69.16^{a} \pm 1.35$	$2.83^{a} \pm 0.46$	$1.66^{a} \pm 0.21$
Cycling Group	Heifers	$8.46^{ab} \pm 0.98$	$24.66^{a} \pm 2.38$	$71.66^{a} \pm 1.92$	$2.33^{a} \pm 0.49$	$1.33^{a} \pm 0.33$
	Lactating cows	$7.23^{b} \pm 0.57$	$18.50^{b} \pm 1.05$	$77.83^{b} \pm 1.35$	$2.33^{a} \pm 0.49$	$1.33^{a} \pm 0.42$

and physical condition. The TLC of 10-13 months heifers of present experiment was similar to Jersey heifers, lactating cows and 3-4 years Jersey female cattle (Schalm *et al.*, 1975).

The TLC of lactating cows of present experiment was similar to the TLC of Orissa cattle (Mishra and Biswal 1961). The TLC of cycling animal was comparatively lower than the TLC of noncycling heifers. The same trend has also been reported by Kumar *et al.* (1991) in non descript rural buffaloes. The lower number of WBC counted were also supported by Gupta *et al* (1996) who reported lower number of WBC in milking cows than that of heifers and dry cows. The lower value of TLC in lactating cows during present study might be due to the fact that WBC count decreases during lactation (Paape *et al.* 1974).

It is also reported that fear, excitement, increased rate and force of the heart tends to elevate WBC count by flushing sequestered cells into blood (Schalm *et al.* 1975). This might be the cause behind the increased number of WBC in heifers than that of cows, because heifers remain free for grazing in the farm while the cows are accustomed to be tied at the time of milking and feeding and so the heifer are more excited or sustain physiological stress than cows during the restraining for blood sampling.

The percent of lymphocytes obtained was similar to the range reported by Schalm *et al.* (1975). The lymphocyte percent in 10-13 months, 18-21 months and 27-30 months heifers were similar to the value of lymphocyte percent reported by Kumar *et al.* (1990) in Murrah buffalo heifers of 12 months, 20-24 months and 25-30 months respectively. The lymphocyte percentage of lactating cows is more than that of any group of heifers and the same finding was reported by Gupta *et al.* (1996) who also found a greater percent of lymphocyte in milking Sahiwal cows than the Sahiwal heifers and non milking cows.

The percentage of neutrophil obtained is within the normal range reported by Schalm *et al.* (1975). In present findings the percent of Neutrophils obtained in lactating cows is less than that of other groups which was also reported by Schalm *et al.* (1975) and this may be due to the fact that neutrophil number decreases during lactation period (Paape *et al.* 1974).

The circulating percentage of Eosinophil of different groups of animals falls within the range reported by Swenson and Reece (1996). The Eosinophil percent of different groups are similar to each other, and the same value was also reported by Kumar *et al* (1990) in Murrah heifer.

The monocyte percentage obtained in different groups are similar to each other and the same value was also reported by Kumar *et al* (1990) in Murrah buffaloes.

In present investigation we could not see the Basophils in the blood smear prepared from the experimental animals. Some investigators have already reported that the value of Basophils in growing animals and adult cows are very less (0.1to 1.1%) in different breeds of cattle (Holman et al 1956, Gupta *et al* 1996). The Basophil percentage might be too low to count in few fields focused under microscope to count the different leucocytes. The other investigators (Greatorex, 1954; Kaneko and Mills 1970 Sridhar *et al.* 1988) also could not detect the Basophil in their experimental animals (cattle) of different age groups. It has been reported that Basophils may occur in number too low to allow detection of any influence of age (Schalm *et al.* 1975).

4.2 Circulating Biochemical Concentration of the Experimental Animals

4.2.1 Total Serum Protein (TSP), Albumin and Globulin

The total serum protein, albumin and Globulin value (Mean ± SE) present in different group of crossbred animals are presented in table-4. The value of total serum protein in noncycling heifers of 10-13 months, 18-21 months, 27-30 months, cycling heifers

and cycling lactating cows were 4.06 ± 0.13 g/100 ml, 5.43 ± 0.27 g/100 ml, 6.20 ± 0.18 g/100 ml, 7.82 ± 0.28 g/100 ml, and 9.34 ± 0.36 g/100 ml respectively. The TSP in lactating cow was significantly ($P<0.01$) higher than the TSP of other groups and the level in 10-13 months heifers was significantly lower ($P<0.01$) than that of other groups. The TSP level of 18-21 months heifers was lower ($P<0.05$) than that of 27-30 months heifers. Individually the total serum protein value ranged between 3.6 g/100ml to 4.40 g/100 ml in 10-13 months heifers, 4.60 g/100 ml to 6.60 g/100ml in 18-21 months heifers, 5.55 g/100 ml to 6.80 g/100ml in 27-30 months heifers, 7.11 g/100 ml to 8.66 g/100 ml in cycling heifer and 8.44 g/100 ml to 10.90 g/100 ml in cycling lactating cows.

The serum albumin level (mean ± SE) was 1.46 ± 0.11 g/100ml in 10-13 months heifers, 1.90 ± 0.11 g/100 ml in 18-21 months heifers, 2.32 ± 0.09 g/100 ml in 27-30 months heifers, 3.02 ± 0.12 g/100 ml in cycling heifers and 3.71 ± 0.15 g/100 ml in cycling lactating cows. The serum albumin level in 10-13 months heifers was significantly lower than cycling lactating cows, cycling heifers, non cycling heifers of 27-30 months ($P<0.01$) and non cycling heifers of 18-21 months ($P<0.05$). The serum albumin level of 18-21 months heifer is lower ($P<0.05$) than that of 27-30 months heifer and it is significantly ($P<0.01$) lower than that of cycling heifers and cycling lactating cows. The serum albumin level of cycling lactating cow is significantly higher ($P<0.01$) than that of any other group.

Individually the serum albumin level ranged between 1.00 g/100 ml to 1.80 g/100 ml in 10-13 months heifers, 1.60 g/100 ml to 2.40 g/100 ml in 18-21 months heifers, 2.00 g/100 ml to 2.66 g/100 ml in 27-30 months heifers, 2.66 g/100 ml to 3.33 g/100 ml in cycling heifers and 3.33 g/100 ml to 4.44 g/100 ml in cycling lactating cows.

The serum globulin level (mean ± SE) in 10-13 months heifer, 18-21 months heifer, 27-30 months heifer, cycling heifer and cycling lactating cows were 2.60 ± 0.11 g/100 ml, 3.53 ± 0.18 g/100 ml, 3.88 ± 0.15 g/100 ml, 4.81 ± 0.19 g/100 ml and 5.63 ± 0.27 g/100 ml respectively. The serum globulin value of 10-13 months non cycling heifer is significantly lower ($P<0.01$) than that of any other group of crossbred animals. The level of serum globulin in cycling lactating cow is significantly higher ($P<0.01$) than that of any other group. Serum globulin level in 18-21 months heifers and 27-30 months are similar ($P>0.05$) to each other.

Individually the serum globulin level ranged between 2.20 g/100 ml to 3.00 g/100 ml in 10-13 months heifers, 2.80 g/100 ml to 4.20 g/100 ml in 18-21 months heifer, 3.33 g/100 ml to 4.40 g/100 ml in 27-30 months heifer, 4.23 g/100 ml to 5.55 g/100 ml in cycling heifers and 4.80 g/100 ml to 6.46 g/100 ml in cycling lactating cows.

During the present experiment it has been recorded that the TSP, Albumin and Globulin have an increasing trend as per advancing age. A similar trend was also reported in crossbred heifers

Table - 4

Total Serum Protein, Serum Albumin and Serum Globulin (Mean ± SE) in Different groups of Crossbred Animals (Heifers & Cows)

Animal Group		Total Serum Protein (g%)	Serum Albumin (g%)	Serum Globulin (g%)
Non Cycling Group	10-13 Months	$4.06^{a} \pm 0.13$	$1.46^{a} \pm 0.11$	$2.60^{a} \pm 0.11$
	18-21 Months	$5.43^{b} \pm 0.27$	$1.90^{b} \pm 0.11$	$3.53^{b} \pm 0.18$
	27-30 Months	$6.20^{c} \pm 0.18$	$2.32^{c} \pm 0.09$	$3.88^{b} \pm 0.15$
Cycling Group	Heifers	$7.82^{d} \pm 0.28$	$3.02^{d} \pm 0.12$	$4.81^{c} \pm 0.19$
	Lactating cows (1^{st} to 4^{th} lactation)	$9.34^{e} \pm 0.36$	$3.71^{e} \pm 0.15$	$5.63^{d} \pm 0.27$

(Manowar and Singh, 2002; Patil *et al.* 2000 and Gaikwad *et al.* 1992). Such increasing level of total serum protein with advancing age appears to be associated with the demand of proteins for the tissue of growing animals exhibiting optimum metabolism and higher protein synthesis.

The circulating level of protein depends upon nutrient supply, age, managemental condition and internal physiological mechanism responsible for assimilation of protein in the body system. There are several dairy breeds maintained in varied agroclimatic zones of the country and abroad. The different breeds are adapted to their own climatic conditions and exhibit their optimum reproductive and productive potential in the climate where they have born and maintained since last several generations. When the animals are replaced from temperate to tropical region and vice-versa they require certain period of time/generation to adapt in changed environment. During the course of adaptation the vital intrinsic organs responsible for regulation of normal body composition work under severe stress and can not maintain the normal biochemical constituents of blood till they adapt fully to their new environment (Akopjan, 1941; Bonsma *et al.* 1940; Manresa *et al.* 1940). The experimental animals of present investigation are crossbred (Hariana × Friesian) of F_3 generation. The blood biochemical constituents may be at par with the biochemical constituents reported in crossbred animals in tropical climate. By overviewing the values of TSP, serum albumin and serum

globulin in different age group the sequential increase in mean total serum protein albumin and globulin was observed (table – 4). This sequential increase indicates that the mechanism of synthesis of total serum protein, albumin and globulin are identical and age related in growing heifers of preset experiment. The non cycling heifers neither exhibited the behavioural estrus nor were having corpus luteum on their ovary during the period of investigation. Hence it is assumed that those heifers though have achieved the chronological age of puberty yet they have not resumed the cyclic rhythm as it has been observed that the age of puberty of F_1, F_2 and F_3 generation crossbred (Hariana × HF) heifers maintained in cattle farm, Pusa is 20-26 months (Singh, 1990).

The increasing trend of serum protein with the advancement of age was also reported in Gir × HF heifers (Patil *et al.* 2000) and in Jersey × Red Khandhari heifers (Gaikwad *et al.* 1992). Moreover, the majority of investigators so far have reported increasing trend of serum protein in growing heifers (Prabha et al, 2000; Manower and Singh 2002).

In present experiment the higher total serum protein level in cycling animals than noncycling animals are in conformity with the earlier report of TSP in cycling animals than that of non cycling animals (Vhora *et al.* 1995; Shrivastava and Kadu, 1995; Ali *et al.* 1991, Dutta *et al.* 1988). Moreover, the higher TSP, albumin and

globulin in cycling animals than noncycling animals are in agreement with the earlier reports (Arosh *et al.* 1998, Ramakrishna 1997, Kavani *et al.* 1987, Manowar and Singh 2002).

The serum protein level of lactating cows of present experiment was higher than the level reported in lactating cows earlier (Kulkarni *et al.* 1983; Shrikhande and Sarode 1999; Gaikwad *et al.* 1992). However, the value of serum protein in lactating cows is similar to the value reported in cows and buffaloes (Kumar *et al.* 2001) and lactating cows of different breeds (Gandotra *et al.* 1992; Manowar and Singh 2002).

The linear increase in TSP associated with increase in both serum albumin and globulin with advancing age in animals may be due to the fact that high metabolic rate of the younger animals results high rate of cellular reaction using protein as a substrate that results into lower level of total serum protein, serum albumin and serum globulin in younger animals as similar observations has been reported in growing crossbred heifers (Manowar and Singh, 2002).

The significantly higher serum protein in lactating dairy cows than the cycling heifers as well as higher protein concentration in cycling heifers than non cycling heifers suggest that threshhold levels of serum protein are different at various ages and reproductive states of dairy cows. The value of serum protein is also indicative of

the reproductive status of the cows as significantly higher level of total serum protein were recorded in cyclic than noncyclic cows (Tandle *et al.* 1997; Arosh *et al.* 1998; Ramakrishna, 1997). Through an earlier observation it has been reported that a certain elevated level of protein in circulation is necessary for the setting of the intrinsic mechanism necessary for the expression of optimal reproductive potential in cows (Patil and Deshpande, 1979). The higher elevation of the serum protein towards advancing age of the crossbred heifers of present investigation confirm that requirement for higher protein concentration towards the onset of puberty and cyclicity of crossbred heifers are essential as reported above.

4.2.2 Serum Uric Acid and Serum Creatinine

The mean ± SE values of serum uric acid and serum creatinine in different age group of crossbred heifers and lactating cows are presented in table-5. The serum uric acid and serum creatinine concentration was 1.04 ±0.07 mg/100ml and 1.77±0.40 mg/100 ml in 10-13 months, 1.16±0.12 mg/100ml and 1.77±0.33 mg/100ml in 18-21 months heifers, 1.35 ±0.18 mg/ 100 ml and 1.88±0.36 mg/100ml in 27-30 months heifers, 1.67±0.21 mg/100 ml and 1.99±0.17 mg/100ml in cycling heifers and 1.52±0.28 mg/100ml and 1.55±0.28 mg/100ml in cycling lactating cows respectively.

The mean concentration of serum uric acid in cycling lactating cow is similar ($P>0.05$) to the serum uric acid value of other

Table – 5

Serum Uric Acid and Serum Creatinine (Mean ± SE) level in Different groups of Crossbred Animals (Heifers and Cows)

Animal Group		Uric acid (mg/100ml)	Creatinine (mg/100 ml)
Non Cycling Group	10-13 Months	$1.04^{a} \pm 0.07$	$1.77^{A} \pm 0.40$
	18-21 Months	$1.16^{a} \pm 0.12$	$1.77^{A} \pm 0.33$
	27-30 Months	$1.35^{a} \pm 0.18$	$1.88^{A} \pm 0.36$
Cycling Group	Heifers	$1.67^{a} \pm 0.21$	$1.99^{A} \pm 0.17$
	Lactating cows (1^{st} to 4^{th} lactation)	$1.52^{a} \pm 0.28$	$1.55^{A} \pm 0.28$

groups. However, the serum creatinine concentration in different age group of crossbred heifers and lactating cows are not different ($P>0.05$) from each other.

Individually the serum uric acid value ranged from 0.83 mg/100ml to 1.25 mg/100ml in 10-13 months heifers, from 0.85 mg/100ml to 1.66 mg/100ml in 18-21 months heifers, 0.83 mg/100ml to 1.90 mg/100ml in 27-30 months heifers, from 1.25 mg/100ml to 2.50 mg/100ml in cycling heifers and from 0.62 mg/100ml to 2.50 mg/100 ml in cycling lactating cows.

The serum creatinine concentration ranged from 0.66 mg/dl to 2.66 mg/dl in 10-13 months heifers, 0.66 mg/dl to 2.66 mg/dl in 18-21 months heifers, 0.66 mg/dl to 3.33 mg/dl in 27-30 months heifers, 1.32 mg/dl to 2.66 mg/dl in cycling heifers and 0.66mg/dl to 2.66 mg/dl in cycling lactating cows.

The value of serum uric acid and serum creatinine increases from lower age group to higher age group during observation except the lactating cows which had lower concentration than that of cycling heifers although the difference is non significant ($P>0.05$).

Until recently there is very little published record available on the uric acid level in the different growing ages and reproductive status of crossbred cattle.

However, the value of serum uric acid and serum creatinine in crossbred heifers and lactating cows observed during

present investigation is within the range of serum uric acid and creatinine in cows born and maintained in temperate climate (Swenson and Reece, 1996).

Uric acid is formed from the degradation of purines, adenine and guanine. With the exception of man and higher apes mammals convert uric acid to allantoin by action of enzyme uricase (Swenson and Reece 1996). The ingestion of foods high in nucleo protein such as glandular organs produces a marked increase in uric acid (West et al, 1966).

In the present estimation the increasing trend as per age of heifers was seen with the serum uric acid and serum creatinine. Both the constituents increases with age and the values are found to be decreased in the lactating cows than that of cycling heifers. Although the difference is not much significant among the different groups but it appears that there is some relation between the uric acid and creatinine level in the blood serum of the crossbred cattle and so it needs some further specialized investigations establishing a relation between uric acid and creatinine in the growing animals and in different reproductive states of the animals.

The non significant increasing trend of uric acid with age found during present study is in agreement with the gradual increase in the uric acid concentration in Black Bengal kids (Kalita and Mahapatra, 1999) and cross bred (Gir × HF) female calves (Patil *et al.* 2000).

Our present findings indicate that the cycling animals have comparatively higher level of serum uric acid than that of non cycling animals which is in line with the observation of Meli et al (1984) who reported the higher value of uric acid in regular breeding cows than cows having repeat breeding problems

During present investigation the nonsignificant increasing trend was observed with the serum creatinine also. The value of serum creatinine in the lactating cows is similar to the value reported earlier in cows. (Kulkarni *et al.* 1984a; Mengi *et al.* 2001). Moreover, the non significant increasing trend was also been estimated by Patil et al (2000) in Gir x HF heifers.

Similar increasing trend of creatinine has also been reported in Black Bengal Kids (Kalita and Mahapatra, 1999). The non significant difference observed on creatinin levels of cycling and noncycling animals of present experiment is similar to the level of serum creatinine in cycling and anoestrus cows (Arosh *et al* 1998).

4.2.3 Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT).

Since more than six decades attempts have been made to introduce the values of enzymes in domestie animals with a view to establish normal activity of different enzymes during different states of health and production. Estimation of serum concentration of different enzymes activity in normal metabolism have become increasingly useful to relate the levels of the enzymatic activity with the necrosis, degeneration or regeneration of tissue cells which are

supposed to be the rich source of these enzymes. During the course of formation of these enzymes and during its activity in the tissue cells some fractions of the enzyme spill over and / or escape from the cells into the blood stream. The quantity of the enzyme activity in the serum has been considered directly proportional to its rate of formation and the activity in the synthesizing cells. Thus the activities of enzymes in the serum determine the physiological and pathological status of the cells in which the enzymes are synthesized and remain active.

The mean ± SE values of SGOT and SGPT activities in different age group of crossbred heifer has been presented in table 6. The activity of SGOT and SGPT in 10-13 months heifers, 18-21 months heifers, 27 to 30 months heifers, cycling heifers and cycling lactating cows were 47.33±5.25 mIu/ml and 26.16 ± 3.51 mIu /ml, 40.67 ± 4.58 mIu /ml and 19.66 ± 1.98 mIu/ml, 50.17 ± 2.94 mIu/ml and 24.83 ± 3.22 mIu/ml, 38.16 ± 4.23 mIu/ml and 21.83 ± 3.19 mIu/ml and 40.17 ± 6.68 mIu/ml and 20.17 ± 2.48 mIu/ml respectively.

Individually the SGOT and SGPT activity ranged from 34 mIu/ml to 61 mIu/ml and 18 mIu/ml to 39 mIu/ml in 10 to 13 months heifers, 24 mIu/ml to 54 mIu/ml and 13 mIu/ml to 24 mIu/ml in 18 to 21 months of heifer, 40 mIu/ml to 61 mIu/ml and 14 mIu/ml to 33 mIu/ml in 27-30 months heifers, 24 mIu/ml to 47 mIu/ml and 14 mIu /

Table – 6

SGOT, SGPT and Alkaline Phosphatase level (Mean ± SE) in different group so corssbred animals (Heifers and Cows)

Animal Group		SGOT (mIu/ml)	SGPT (mIu/ml)	Alkaline Phosphatase (KA/100ml)
Non Cycling Group	10-13 Months	47.33^{A} ± 5.25	26.16^{α} ± 3.51	16.11^{a} ± 1.24
	18-21 Months	40.67^{A} ± 4.58	19.66^{α} ± 1.98	19.07^{a} ± 3.67
	27-30 Months	50.17^{A} ± 2.94	24.83^{α} ± 3.22	19.25^{a} ± 4.18
Cycling Group	Heifers	38.16^{A} ± 4.23	21.83^{α} ± 3.19	30.00^{b} ± 3.16
	Lactating cows (1^{st} to 4^{th} lactation)	40.17^{A} ± 6.68	20.17^{α} ±2.48	35.74^{b} ± 4.97

ml to 33 mIu/ml in cycling heifers and 21 mIu/ml to 61 mIu/ml and 13 mIu / ml to 28 mIu/ml in cycling lactating cows respectively. The level of activity of SGOT and SGPT in different age groups of crossbred heifers were statistically similar ($P>0.05$) to each other.

Among the enzymes the transaminases are the primary enzymes which are normally concerned with the transfer of α-amino group of either aspartic acid or alanine to α-ketoglutaric acid and resulting in formation of oxaloacetic acid and pyruvic acid respectively. GPT is much more abundant in liver than in other tissues and consequently an elevated GPT coincide with the liver disease in contrast to the elevated GOT which may rise due to many cases. Several types of GOT having identified in serum. These are believed to originate in different tissues but they may differ also with respect to source within the cell (Cornelius *et al.* 1959; Oser, 1954). The values of SGOT and SGPT activity obtained in crossbred heifers of F_3 generation during present investigation will determine the normal SGOT and SGPT activity in different aged crossbred heifers born and maintained in tropical climate.

The level of SGOT and SGPT activity detected during the present study in crossbred animals are within the normal range reported in cattle (Samanna and Ramaswamy, 1976). Moreover, the SGOT level is similar to the value in Hariana heifers and lactating cows (Murtuza *et al.* 1980). The present value of SGOT and SGPT

activity in 18 to 21 months crossbred heifers are similar to the value reported in 16-20 months Hariana and Sahiwal heifers (Payne and Maitra, 1980).

The SGOT activity was found to be higher in cycling cows than in noncycling cows (Arosh *et al.* 1998). However, in present study no any significant difference in SGOT level was estimated between cycling and non cycling animals. Moreover, the similar level of SGOT activity was also reported between primary infertile heifers and normal cyclic heifers (Sharma *et al.* 1986) and Rathi and Sahiwal heifers and cow (Singh *et al.* 1973). Earlier reports revealed that age of the animals have some effect on the level of SGOT and SGPT activity. SGPT level in buffaloes (Rathee *et al.* 2002), SGOT and SGPT level in camels (Kataria *et al.* 1991), SGOT level in crossbred female calves (Atak *et al.* 2000) and Black Bengal goats (Behera *et al* 1993) were showing significant difference between different age groups, although no any trend could be establish with the increasing age. However, in present experiment age has no any significant effect on the activity of SGOT and SGPT. Our observation of level and pattern of SGOT and SGPT in crossbred animals of the present experiment is agree with earlier report of SGOT level in Jersey cows (Roussel *et al.* 1982) and Murrah buffalo calves and heifers (Rathee *et al.* 2000) and SGPT level in crossbred calves (Atak *et al.* 2000) and Black Bengal goats (Behera *et al.* 1993). The SGOT and SGPT activity

detected at various age groups of crossbred heifers during present investigation falls under the range of SGOT and SGPT activity reported in cattle (Samanna and Ramaswamy, 1976; Pyne and Maitra, 1980).

4.2.4 Serum Alkaline Phosphatase activity

Very little published information is available about the serum alkaline phosphatase activity in different reproductive states of the crossbred animals. Crossbreeding is being adopted since last few decades to enhance the milk production and so some changes in the various metabolic profiles of newly evolved crossbred animals are expected. There is a great need of systematic approach to study the enzymatic activity in crossbred animals. This chapter have dealt with the normal level of serum alkaline phosphatase activity in crossbred heifers of different age group and reproductive states.

The activity (Mean ± SE) of serum alkaline phosphatase in crossbred heifers of different age groups has been presented in table 6. The serum alkaline phosphatase activity was 16.11±1.24 KA/100ml, 19.07 ± 3.67 KA/100ml, 19.25 ± 4.18 KA/100 ml, 30.00±3.16 KA/100ml and 35.74±4.97 KA/100ml in 10 to 13 months heifers, 18 to 21months heifers, 27-30 months heifers, cycling heifers and cycling lactating cows.

Individually the serum alkaline phosphatase activity ranged from 12.22 KA/100 ml to 19.99 KA/100ml in 10 to 13 months

heifers, 12.22 KA/100 ml to 35.55 KA/ 100 ml in 18 to 21 months heifers, 13.33 KA/100 ml to 40.00 KA/100ml in 27 to 30 months heifers, 23.33 KA/ 100 ml to 42.22 KA//100 ml in cycling heifers and 20.00 KA/100 ml to 55.55 KA/100 ml in cycling lactating cows.

The alkaline phosphatase activity in cycling heifer ($P<0.05$) and cycling lactating cows ($P<0.01$) was significantly higher than that of noncycling heifers. The activity of serum alkaline phosphatase between cycling heifers and cycling lactating cows and also between non-cycling heifers of different ages were similar ($P>0.05$).

The serum alkaline phosphatase is a relatively non specific and stable enzyme hydrolyzing almost all orthophosphoric monoesters. In farm animals efforts are being made to use these enzymes levels as genetic markers for the improvement of production and disease traits. It is known that enzyme activities are controlled by both genetic and nongenetic factors. In some cases genetic parts are more while in other non genetic parts are dominating (Mazumder and Mazumder, 1985) Investigations have to be made to find out the relative contribution in each case.

A wide variation of alkaline phosphatase activity in normal healthy *Bos taurus* cows was reported by Allcroft and Folley (1941) and Crookshank *et al.* (1952). They however, did not report the normal values of alkaline phosphatase activity breed wise. Variations

of serum alkaline phosphatase activity in Indian breeds of cattle were reported by various workers (Goswami *et al.* 1971; Singh *et al.* 1973; Pandiya *et al.* 1977; Atak *et al.* 2000). The lower variation might be due to either breed differences or due to the different number of animals considered by them.

The serum alkaline phosphatase activity estimated during present experiment in 10 to 13 months and 18 to 21 months heifers are similar to the level reported in 12 months and 24 months old crossbred cattle respectively (Mazumder and Mazumder, 1985). The effect of age on the serum alkaline phosphatase activity has also been reported. The enzyme activity was higher during the most active period of development. It reached peak values on attaining adult age (Mazumder and Mazumder, 1985).

The serum alkaline phosphatase activity in anestrus heifer is more than that of normal cycling heifers (Sharma et al, 1986 and lactating cows (Murtuza et al 1980). However, in present study the serum alkaline phosphatase activity in cycling animals were higher than that of noncycling animals. Moreover, the higher concentration of serum alkaline phosphatase activity was estimated in normal cycling buffaloes than that of repeat breeding buffaloes (Gandotra et al, 1993) and the enzyme activity was estimated higher also in normal cycling crossbreed cows than that of anoestrus crossbred cows (Arosh *et al.* 1998). Hormonal imbalance and deranged enzymatic action affect the normal reproductive behaviour of the

animal and cause physiological alteration. The concentration of this enzyme is indicative of the level of physiological activity of the tissues. In this investigation, the lower concentration of serum alkaline phosphatase in noncycling heifers might be associated with reduced physiological activity of reproductive organs in anoestrus animals (Arosh *et al.* 1998).

4.2.5 SERUM SODIUM AND POTASSIUM

The serum level of Sodium and Potassium estimated in different age groups of crossbred heifers and lactating cows has been presented in table 7. The level (Mean ± SE) of sodium and potassium respectively was 144.23±1.21 mEq / lit and 4.51 ± 0.44 mEq/lit in 10 to 13 months heifers, 138.90 ±1.94 mEq / lit and 3.53 ± 0.17 mEq/lit in 18 to 21 months heifers, 138.27 ± 4.06 mEq/ lit and 3.67 ± 0.47 mEq/lit in 27-30 months heifers, 147.55 ± 3.29 mEq/lit and 4.37 ± 0.24 mEq / lit in cycling heifers and 138.77±5.08 mEq / lit and 3.96 ± 0.45 mEq / lit in cycling lactating cows.

Individually the level of sodium and potassium respectively ranged between 142.20 mEq/lit to 149.00 mEq/lit and 3.01 mEq / lit to 6.30 mEq /lit in 10-13 months heifers, 131.4 mEq /lit to 144.0 mEq / lit and 2.87 mEq / lit to 4.20 mEq / lit in 18-21 months heifers, 118.60 mEq /lit to 145.80 mEq/lit and 2.52 mEq/lit to 5.15 mEq/lit in 27 to 30 months heifers, 133.20 mEq./lit to 156.40 mEq/lit

Table –7

Serum Sodium and Serum Potassium (Mean ± SE) in different groups of crossbred animals (Heifers and Cows)

Animal Group		Sodium (mEq/L)	Potassium (mEq/L)
Non Cycling Group	10-13 Months	$144.23^{a} \pm 1.21$	$4.51^{A} \pm 0.44$
	18-21 Months	$138.90^{a} \pm 1.94$	$3.53^{A} \pm 0.17$
	27-30 Months	$138.27^{a} \pm 4.06$	$3.67^{A} \pm 0.47$
Cycling Group	Heifers	$147.55^{a} \pm 3.29$	$4.37^{A} \pm 0.24$
	Lactating cows (1^{st} to 4^{th} lactation)	$138.77^{a} \pm 5.08$	$3.96^{A} \pm 0.45$

and 3.57 mEq/lit to 5.18 mEq/lit in cycling heifers and 124.20 mEq/lit to 154.80 mEq/lit and 2.80 mEq/lit to 5.74 mEq/lit in cycling lactating cows.

The value of sodium and potassium ions estimated in different age groups of crossbred heifers and cows are statistically similar ($P>0.05$) to each others.

The level of sodium and potassium estimated in crossbred animal of present investigation are within the range reported already in cattle (Kaneko *et al.* 1997). The value of sodium and potassium obtained in lactating cows during present study is similar to the value detected in Gir and Jersey cows (Kulkarni *et al.* 1984) and non descript Kashmiri cows (Sharma *et al.* 1995). The difference in the sodium and potassium concentration between the lactating and other groups of animals was not significant and the same finding was also reported in Murrah buffaloes where the difference in sodium as well as potassium concentration in lactating and dry buffaloes were not significant (Kulkarni *et al.* 1984a). The effect of age on the level of sodium and potassium ion concentration could not be detected in crossbred animals. A similar finding has also been reported by Shrikhande and Sarode (1999) who did not observe any significant difference between different age group of cows. The difference between the level of sodium and potassium among the cycling and non cycling animals were not significant. The same finding was also

reported in Jersey- Sahiwal crossbred cows (Agarwal *et al.* 1982) and in the Yak (Singh *et al.* 1999). However, a lower concentration of sodium ion was reported in anoestrus cows than the normal cyclic cow although the difference in potassium concentration was nonsignificant between anoestrus and cyclic cows. In the case of heifers the potassium concentration was significantly higher in normal cycling heifers than that of anoestrus heifers although the level of sodium ion was similar in cycling and noncycling heifers (Kumar *et al.* 1986).

The value of sodium and potassium estimated in the serum of different age group of crossbred animals of present experiment are similar to the serum level of sodium and potassium in cows (Kaneko *et al.* 1997).

Chapter – 5

Summary and Conclusion

SUMMARY AND CONCLUSION

SUMMARY

The hemograms and some of the biochemical constituents of blood was determined in crossbred (Hariana × Holstein Friesian) heifer and cows. The experimental animals were selected from the animal herd of Animal Production Research Institute, RAU Pusa, Samastipur. Six non cycling crossbred heifers at each group of 10-13 months, 18-21 months, 27-30 months, six cycling crossbred heifers and six cycling lactating crossbred cows were selected. All the experimental animals were apparently healthy and were maintained in the herd of the respective age group. They were being offered feed and fodders twice daily as was practiced in the farm. All the blood samples from the animals were collected between 6 a.m. to 7 a.m. from the jugular vein and were processed for determining BCT, Hb, PCV, TEC, MCV, MCH, MCHC, TLC, DLC and ESR. Estimation of total serum protein, serum albumin, serum globulin, uric acid, creatinine, SGOT, SGPT, alkaline phosphatase, sodium and potassium was done from the blood serum by using standard laboratory technique.

In non cycling heifers of 10-13 months, 18-21 months, 27-30 months; cycling heifers and cycling lactating cows, the TEC (million / mm^3) was 7.06 ± 0.47, 6.43 ± 0.76, 8.20 ±0.53, 8.35 ± 0.39

and 5.67 ± 0.23; Hb (g/dl) was 12.03 ± 0.80, 11.92 ± 0.78, 14.66 ± 0.54, 13.33 ± 0.98 and 11.50 ± 0.75; PCV (%) was 32.33 ± 2.40, 33.83 ± 2.39, 39.33 ± 1.08, 36.67 ± 2.11 and 29.08 ± 3.52 ; MCV (μ^3) was 47.21 ± 5.21, 55.24 ± 6.03, 49.33 ± 4.39, 44.43 ± 3.33 and 50.57 ± 3.87 ; MCH (pg) was 17.53 ± 1.81, 19.64 ± 2.37, 18.31 ± 1.60, 16.13 ± 1.40 and 20.20 ± 0.53; MCHC (%) was 37.39 ± 0.95, 35.31 ± 0.93, 37.42 ± 1.82, 36.58 ± 2.64 and 40.65 ±1.91 ; BCT (minute) was 6.50 ± 0.43, 8.00 ± 0.63, 6.66 ± 0.61, 6.50 ± 0.41 and 6.00 ± 0.38 and ESR (mm/hr) was 0.29 ± 0.04, 0.46 ± 0.12, 0.48 ± 0.11, 0.37 ± 0.12 and 0.58 ± 0.12 respectively.

Further estimation reveals that in the non cycling heifers of 10-13 months, 18-21 months, 27-30 months, cycling heifers and cycling lactating cows the value of TLC (thousand / mm^3) was 8.82 ± 0.83, 10.13 ± 0.53, 9.37 ± 1.15, 8.46 ± 0.98 and 7.23 ± 0.57; Neutrophils (%) was 26.5 ± 1.72, 26.83 ± 1.74, 26.33 ± 1.40, 24.66 ± 2.38 and 18.50 ± 1.05; lymphocytes (%) was 70.00 ± 1.73, 68.16 ± 1.62, 69.16 ± 1.35, 71.66 ± 1.92 and 77.83 ± 1.35; Eosinophils (%) was 2.50 ± 0.34, 3.16 ± 0.40, 2.83 ± 0.46, 2.33 ± 0.49 and 2.33 ± 0.49; Monocytes (%) was 1.00 ± 0.36, 1.83 ± 0.16, 1.66 ± 0.21, 1.33 ± 0.33 and 1.33 ± 0.42 respectively.

A gradual increase in the concentration of total serum protein, serum albumin and serum globulin was observed from lower age group to higher age group. The TSP (g%), albumin (g%) and

globulin (g%) was 4.06 ± 0.13, 1.46 ± 0.11 and 2.60 ± 0.11 in 10-13 months; 5.43 ± 0.27, 1.90 ± 0.11 and 3.53 ± 0.18 in 18-21 months; 6.20 ± 0.18, 2.32 ± 0.09 and 3.88 ± 0.15 in 27-30 months, 7.82 ± 0.28, 3.02 ± 0.12 and 4.81 ± 0.19 in cycling heifers and 9.34 ± 0.36, 3.71 ± 0.15 and 5.63 ± 0.27 in cycling lactating cows respectively.

The value of serum alkaline phosphates (KA / 100ml), SGOT (mIu/ml) and SGPT (mIU/ml) in 10-13 months heifers was 16.11 ± 1.24, 47.33 ± 5.25, and 26.16 ±3.51; in 18-21 months 19.07 ± 3.67, 40.67 ± 4.58 and 19.66 ± 1.98; in 27-30 months heifers 19.25 ± 4.18, 50.17 ± 2.94 and 24.83 ± 3.22 ; in cycling heifers 30.00 ± 3.16, 38.16 ± 4.23 and 21.83 ±3.19 ; in lactating cows 35.74 ± 4.97, 40.17 ± 6.68 and 20.17 ± 2.48 respectively.

The serum concentration of uric acid (mg / 100 ml), creatinine (mg/100ml), sodium (mEq /L) and potassium (mEq /L) in 10-13 months heifers was 1.04 ± 0.06, 1.77 ± 0.40, 144.23 ± 1.21 and 4.51 ± 0.44, in 18-21 months heifers was 1.16 ± 0.12, 1.77 ± 0.33, 138.90 ± 1.94 and 3.53 ± 0.17; in 27-30 months heifers 1.35 ± 0.18, 1.88 ± 0.36, 138.27 ± 4.06 and 3.67 ± 0.47; in cycling heifers 1.67 ± 0.21, 1.99 ± 0.17, 147.55 ± 3.29 and 4.37 ± 0.24; in cycling lactating cows was 1.52 ± 0.28, 1.55 ± 0.28, 138.77 ± 5.08 and 3.96 ± 0.45 respectively.

CONCLUSION

1. The value of TEC, hemoglobin and PCV was found to be lower in the lactating cows.

2. The hemoglobin concentration is directly related to TEC if the MCV is unchanged in crossbred animals of tropical climate.

3. There is existence of some intrinsic compensatory mechanism due to which the lower number of erythrocytes may be compensated by increase in the size of the cells.

4. In crossbred heifers the TLC was higher than cows which might be due to the fact that heifers are more sensitive to physiological stress than lactating cows.

5. The increasing trend of serum protein towards advancing age at puberty and during post partum estrous cyclicity recorded during present experiment suggest that the elevated threshold levels of protein concentration in circulation are required to fulfill the demand of the reproductive vital organs to resume reproductive rhythm in both heifers and lactating cows.

6. The observation indicates that the age and parity has no any influence on serum uric acid, serum creatinine, serum

sodium, serum potassium, SGOT and SGPT concentration. However, serum alkaline phosphatase in non cycling heifer indicates reduced physiological activity than cycling animals.

7. A more systemic study including larger number of crossbred animals at different age and stage of reproduction under different climatic conditions are needed to judge the adaptability of crossbred animals of different generations in our tropical climate.

□□□□□

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