CHAPTER IV
RESULTS AND DISCUSSION

Blood, urine and milk samples were collected from 60 Gir cows between 3 and 5 weeks of lactation. The animals were divided into 3 groups namely healthy (I), sub-clinical ketotic (SCK) (II) and clinical ketotic (CK) (III) based on serum Beta-Hydroxybutyric acid (BHBA) concentration. Animals with serum BHBA concentration < 1.2 mM were kept in healthy group whereas animals with serum BHBA concentration 1.2 - 3.0 mM and > 3.0 mM were kept in sub-clinical ketotic (II) and clinical ketotic (III) group, respectively. Body condition score and all hematological parameters (Hb, PCV, TEC, TLC, DLC, MCH, MCV and MCHC), biochemical parameters (BHBA, Glucose, Protein, Albumin, A/G ratio, Cholesterol, Triglycerides, HDL, LDL and NEFA), serum enzymes (AST, ALT and GGT) and anti-oxidant parameters (Catalase and reduced glutathione) were studied and compared in the aforementioned groups. Milk somatic cell count and fat to protein ratio was also estimated in the group of animals. Ketone bodies in urine and milk was detected with Rothera’s test and Ross test, respectively and compared with solid phase single reagent test developed in the department lab. Urine ketone body concentration was estimated using a quantitative modified nitroprusside test developed in department lab using acetoacetate as standard. The results are presented here with suitable discussion.

4.1 Beta-Hydroxybutyric acid (BHBA):

The BHBA is a ketone body synthesized in liver via metabolism of fatty acids. The serum BHBA concentration is considered as a gold standard for detection of ketosis in dairy cows. The mean BHBA concentrations of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups were 0.45 ± 0.02 mM, 1.59 ± 0.13 mM and 5.32 ± 0.51 mM, respectively (Figure 4.1). The mean BHBA concentrations showed a significant ($P < 0.05$) difference among the groups. This finding is in agreement with a recent report that the ketotic animals have significantly increased BHBA concentrations than control group (Cao et al., 2017). In a report, Suthar et al. (2013) stated that the overall herd prevalence for sub-clinical ketosis is approximately 21.8 % ranging from 11.2 to 36.6 percent. Duffield (2000) suggested that, a good thumb rule
for prevalence of sub-clinical ketosis is approximately 2 to 4 times the incidence rate for cows treated for clinical ketosis. In accordance with these reports our study showed that out of sixty animals, forty were categorized as healthy (67 %), twelve as sub-clinical ketotic (20 %) and eight as clinical ketotic (13 %) animals (Figure 4.2).

![Beta-Hydroxybutyric acid concentration](image)

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>SCK</th>
<th>CK</th>
</tr>
</thead>
<tbody>
<tr>
<td>mM</td>
<td>0.45</td>
<td>1.59</td>
<td>5.32</td>
</tr>
</tbody>
</table>

Figure 4.1: Serum Beta-Hydroxybutyric acid concentration (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicate level of significance ($P < 0.05$) among the groups.

![Prevalance](image)

Figure 4.2: Percent healthy, sub-clinical ketotic and clinical ketotic animals based on serum BHBA concentration between three to five weeks of lactation.

**4.2 Age, Parity and Days in Milk:**

Based on serum BHBA concentrations between 21-30 days in milk, animals were divided into clinical or sub-clinical ketotic animals. Animals in clinical ketotic groups were between 6-15 years of age and 2-7 parity (Table 4.1). However, animals in sub-clinical group were between 4-12 years of age and 1-7 parity (Table 4.2). Animals above 6 years and 3rd parity were more susceptible to clinical ketosis whereas subclinical ketosis was recorded in all age group and all parity of animals.
Table 4.1: Name, age (year), parity (number) and days in milk (DIM; day) of clinical ketotic animals.

<table>
<thead>
<tr>
<th>Name</th>
<th>Age (Year)</th>
<th>Parity (No.)</th>
<th>DIM (Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dhruvi</td>
<td>6</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>Dahi</td>
<td>6</td>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>Sati</td>
<td>7</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>Bhagirathi</td>
<td>7</td>
<td>4</td>
<td>29</td>
</tr>
<tr>
<td>Subhadra</td>
<td>8</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>Namrata</td>
<td>8</td>
<td>3</td>
<td>31</td>
</tr>
<tr>
<td>Sobhna</td>
<td>8</td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td>Gunjan</td>
<td>15</td>
<td>7</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 4.2: Name, age (year), parity (number) and days in milk (DIM; day) of sub-clinical ketotic animals.

<table>
<thead>
<tr>
<th>Name</th>
<th>Age (Year)</th>
<th>Parity (No.)</th>
<th>DIM (Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trushna</td>
<td>4</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Ashwini</td>
<td>4</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td>Padmavati</td>
<td>5</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Latika</td>
<td>6</td>
<td>2</td>
<td>23</td>
</tr>
<tr>
<td>Pandadi</td>
<td>7</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>Viveka</td>
<td>7</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>Vibha</td>
<td>8</td>
<td>3</td>
<td>29</td>
</tr>
<tr>
<td>Ridhhi</td>
<td>8</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>Sobhna</td>
<td>9</td>
<td>4</td>
<td>24</td>
</tr>
<tr>
<td>Mala</td>
<td>11</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Kiran</td>
<td>12</td>
<td>7</td>
<td>23</td>
</tr>
</tbody>
</table>

4.3 Body condition score (BCS):

Body condition influences productivity, reproduction, health, and longevity of dairy cattle. Thinness or fatness can be a clue to underlying nutritional deficiencies, health problems, or improper herd management. Body condition scores (BCS) are an indirect estimate of energy balance. A score of 1 represents a very thin cow, while 5 denote an excessively fat cow, and 3 is an average body condition.

All the animals in healthy, sub-clinical ketotic and clinical ketotic group were evaluated for body condition score on a scale of 1-5. The observation recorded
revealed that the BCS of healthy, sub-clinical ketotic and clinical ketotic animals were 3.26 ± 0.06, 2.82 ± 0.13 and 2.71 ± 0.07 respectively. The mean BCS of sub-clinical and clinical ketotic animals were significantly ($P < 0.05$) lower than the mean BCS of healthy animals (Figure 4.3). Our findings are in close agreement with the findings of Marutsova et al. (2015) where they found a BCS of 3.55 ± 0.36, 3.25 ± 0.27 and 2.51 ± 0.31 for control, SCK and CK groups, respectively. We also found a highly significant ($P = 0.001$) negative correlation ($r = -0.40$) between serum BHBA and BCS of the animals. There is general agreement that post partum negative energy balance provokes a change in BCS of animals. The BCS changes were more in ketotic animals as compared to healthy animals.

![Body Condition Score (BCS)](image)

**Figure 4.3:** Body Condition Score (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicate level of significance ($P < 0.05$) among the groups.

### 4.4 Fat to protein ratio:

The percent fat and percent protein values were recorded using automatic milk analyser (Lactoscan, India) and fat to protein ratio was calculated mathematically. The mean fat to protein ratio of healthy, sub-clinical and clinical ketotic animals were 1.22 ± 0.03, 1.29 ± 0.04 and 1.35 ± 0.03, respectively. The mean fat to protein ratio of clinical ketotic group was found significantly ($P < 0.05$) higher than healthy group. However, the mean of sub-clinical ketotic group was not significantly ($P > 0.05$) different than healthy or clinical ketotic group (Figure 4.4). Fat-to-protein ratio has been proposed as a method to diagnose SCK in dairy cows and it was reported that the best cut-off to diagnose subclinical ketosis (BHBA $\geq 1.2$ mM) in dairy cattle in Ontario was FPR $> 1.33$ but the sensitivity and specificity were only 58% and 69%.
respectively (Duffield et al., 1997). We also found a significantly ($P < 0.05$) positive correlation ($r = 0.49$) between serum BHBA and fat to protein ratio.

![Fat to Protein ratio](image)

<table>
<thead>
<tr>
<th></th>
<th>Fat to Protein ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>a</td>
</tr>
<tr>
<td>SCK</td>
<td>ab</td>
</tr>
<tr>
<td>CK</td>
<td>b</td>
</tr>
</tbody>
</table>

Figure 4.4: Fat to protein ratio (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicates level of significance ($P < 0.05$) among the groups.

### 4.5 Somatic cell count (SCC):

Somatic cell count (SCC), primarily composed of leukocytes or white blood cells, is the total number of cells per milliliter in milk. Cell count in the udder increase as the inflammation worsens and it provides an indication of the degree of mastitis in an individual cow. Microscopic evaluation of milk samples for somatic cell count per milliliter of milk elucidated that there was significant ($P < 0.05$) difference between the mean SCC in the milk of healthy (138500.00 ± 69394.22) and ketotic cows. However, sub-clinical ketotic (814428.57 ± 109292.96) cows did not show any significant ($P>0.05$) difference with clinical ketotic (1123500.00 ± 830680.44) animals (Figure 4.5). A highly significant ($P < 0.001$) positive correlation ($r = 0.78$) was found between BHBA and SCC. The probable explanation for increased SCC during early lactation is greater negative energy balance which predisposes dairy cows to udder inflammation.
Results and discussion

Figure 4.5: Somatic cell count/mL milk (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicates level of significance ($P < 0.05$) among the groups.

<table>
<thead>
<tr>
<th>Somatic cell count/mL</th>
<th>Healthy</th>
<th>SCK</th>
<th>CK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>138500.00</td>
<td>814428.57</td>
<td>1123500.00</td>
</tr>
</tbody>
</table>

Figure 4.6: Various inflammatory and secretory cells Newman,s stain X 1000.

4.6 Hematological parameters:

All hematological parameters like hemoglobin (Hb), Packed cell volume (PCV), Total erythrocyte count (TEC), Total leukocyte count (TLC), Differential leukocyte count (DLC), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) were recorded with automated hematology analyzer (Abacus Junior Vet 5, Diatron, Hungary) in the healthy, subclinical ketotic and clinical ketotic animals and the results are as follows. Our results are in accordance with Marutsova et al. (2015) who also found a leukocytosis in cows with subclinical ketosis compared to control group. He also reported that other hematological parameters like TEC, MCH, MCHC, MCV, Monocytes and Granulocytes were close to control values.
4.6.1 Hemoglobin (Hb):

Hemoglobin, the iron-containing oxygen-transport metalloprotein, is found in the red blood cells of all vertebrates and the normal range in cattle is 8-15 g/dL. Hb concentration below normal range results in anaemia. The mean hemoglobin concentrations recorded in healthy, subclinical ketotic and clinical ketotic animals were 11.21 ± 0.33, 11.41 ± 0.38 and 10.77 ± 0.57 g/dL, respectively. The mean hemoglobin concentrations were in the normal range and did not differ significantly ($P > 0.05$) among the group of animals (Figure 4.7).

![Hemoglobin Graph](image)

**Figure 4.7:** Hemoglobin (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicate level of significance ($P < 0.05$) among the groups.

4.6.2 Packed cell volume (PCV):

Packed cell volume, also known as Hematocrit, is the volume percent of RBC in blood and the normal range of PCV in cattle is 24-46 %.

The average PCV of healthy, subclinical ketotic and clinical ketotic animals were 35.17 ± 0.96, 34.76 ± 1.09 and 32.62 ± 1.99 %, respectively. No significant ($P > 0.05$) difference in the mean PCV was found among the group of animals and all the observations were in normal range (Figure 4.8).

![Packed cell volume Graph](image)

**Figure 4.8:** Packed cell volume (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups.
4.6.3 Total erythrocyte count (TEC):

Red blood cells, also known as erythrocyte, are enucleated cells that contain hemoglobin plays vital role in health and disease. Hence, the total erythrocyte count (TEC) is an important parameter in diagnostic hematology. Disturbance in number or function may result in variety of anaemia. The normal TEC in cattle is reported to be 5-10 x 10^6/cmm.

The mean TEC of healthy (7.56 ± 0.20 x 10^6/cmm), subclinical ketotic (7.24 ± 0.31 x 10^6/cmm) and clinical ketotic (7.19 ± 0.34 x 10^6/cmm) animals were in normal range and showed no significant (P > 0.05) difference among the groups (Figure 4.9).

![Figure 4.9: Total erythrocyte count (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicate level of significance (P < 0.05) among the groups.](image)

4.6.4 Total leukocyte count (TLC):

White blood cells (WBCs), also called leukocytes, are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. The normal range of TLC in cattle is 4-12 x 10^3/cmm.

Although the mean TLC of all the group of animals were in normal range, the mean TLC of clinical ketotic animals (10.77 ± 0.57 x 10^3/cmm) was significantly (P < 0.05) lower than the subclinical ketotic (11.41 ± 0.38 x 10^3/cmm) and healthy (11.21 ± 0.33 x 10^3/cmm) animals (Figure 4.10).
Figure 4.10: Total leukocyte count (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicate level of significance (P < 0.05) among the groups.

4.6.5 Differential leukocyte count (DLC):

The leukocytes (WBCs) are classified in two major cell types, namely, Granulocytes (Neutrophils, Eosinophils and Basophils) and Agranulocytes (Monocytes and Lymphocytes).

4.6.5.1 Neutrophils:

The Neutrophils, characterized by polymorph nucleus, are considered as first line of defence. The normal range of Neutrophils percentage in cattle is 15-45 %.

In our study, we found that the mean neutrophils percent of healthy (34.76 ± 1.36 %) sub-clinical ketotic (32.76 ± 4.49 %) and clinical ketotic (34.79 ± 3.95 %) animals were in normal range. We did not observe any significant (P > 0.05) difference in neutrophil percentage among the groups (Figure 4.11).

Figure 4.11: Neutrophils (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicate level of significance (P < 0.05) among the groups.
4.6.5.2 Eosinophils:

Eosinophils contain cytoplasm packed with granules which takes acidic stain (eosin) hence called eosinophils. The cell contains major basic protein which damages the larvae of parasites. Eosinophils are motile and show chemotaxis and phagocytosis. The normal range of eosinophil percent in cattle is 0-15%.

The mean Eosinophil percent of healthy, sub-clinical ketotic and clinical ketotic animals were 4.10 ± 0.37 %, 4.56 ± 1.09 % and 2.61 ± 0.63%, respectively. The observed values were in normal range and showed no significant (P > 0.05) difference among the groups (Figure 4.12).

![Eosinophils](chart.png)

Figure 4.12: Eosinophils (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicate level of significance (P < 0.05) among the groups.

4.6.5.3 Basophils:

Basophils contain basophilic granules and are occasionally seen in healthy animals. The normal range is 0-2% in cattle. Basophils release the histamine resulting in immediate hypersensitivity reaction and also have role in inflammation.

The mean basophils percent of clinical ketotic animals (0.01 ± 0.01 %) were significantly (P < 0.05) lower than the healthy animals (0.08 ± 0.01 %). However, sub-clinical ketotic animals (0.06 ± 0.02 %) showed non-significant (P > 0.05) difference with healthy or clinical ketotic animals (Figure 4.13).
**4.6.5.4 Monocytes:**

The monocytes are large cells with round to kidney-shaped to pseudo-lobulated nucleus and contain no granules. The monokines secreted by monocytes stimulate T-cells, take part in inflammation, act as pyrogen and stimulate formation of acute phase proteins. The normal range of monocyte percent in cattle is 2-8%.

Our study revealed that the monocyte percent of healthy (2.65 ± 0.26 %), sub-clinical ketotic (4.74 ± 0.74 %) and clinical ketotic (2.55 ± 0.50 %) animals were in normal range and there was a significant ($P < 0.05$) difference in mean monocyte percent of sub-clinical ketotic animals as compared to healthy and clinical ketotic animals (Figure 4.14).
4.6.5.5 Lymphocytes:

Lymphocytes contain a large nucleus that occupies almost the total cell and scanty cytoplasm. They are involved in the very important defence mechanism, called immunity. The B-lymphocytes are responsible for humoral immunity and T-lymphocytes are responsible for cell mediated immunity. The normal lymphocyte percent in cattle is 45-75 %.

In our study we found that all the observed mean lymphocyte percentages were in normal range and the difference between the means is shown in figure 4.15. The mean lymphocytes in healthy animals (58.37 ± 1.40 %), sub-clinical ketotic animals (57.89 ± 4.62 %) and clinical ketotic animals (60.00 ± 3.89 %) showed no significant (P > 0.05) difference among the groups.

![Figure 4.15: Lymphocytes (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicate level of significance (P < 0.05) among the groups.](image)

4.6.6 Mean corpuscular volume (MCV):

Mean corpuscular volume (MCV) is a measure of the average volume of a red blood corpuscle. MCV is helpful in classifying the anaemia into microcytic anemia (MCV below normal range), normocytic anemia (MCV within normal range) or macrocytic anemia (MCV above normal range). The normal MCV for cattle is 40-60 fl.

Then MCV for healthy, sub-clinical ketotic and clinical ketotic animals were 46.72 ± 0.72 fl, 48.41 ± 1.73 fl and 45.37 ± 2.05 fl, respectively (Figure 4.16). The means were in normal range and the differences were statistically non-significant (P > 0.05).
Results and discussion

4.6.7 Mean corpuscular hemoglobin (MCH):

Mean corpuscular hemoglobin is the average mass of hemoglobin per red blood cell in a sample of blood. The normal MCH of cattle is 11-17 pg and MCH value is diminished in hypochromic anemias.

The MCH were in normal range for all the group of animals and the observation in sub-clinical ketotic animals (16.68 ± 0.82 pg) is significantly \( (P < 0.05) \) higher than healthy animals (15.16 ± 0.18 pg) and non-significantly \( (P > 0.05) \) higher than clinical ketotic animals (15.62 ± 0.72 pg) is shown in figure 4.17.

4.6.8 Mean corpuscular hemoglobin concentration (MCHC):

The mean corpuscular hemoglobin concentration (MCHC) is a measure of the concentration of hemoglobin in a given volume of packed red blood cells. The reference range of MCHC for cattle is 30-36 %.
Results and discussion

Then MCHC for healthy, sub-clinical ketotic and clinical ketotic animals were 32.63 ± 0.37 %, 32.9 ± 0.80 % and 34.38 ± 0.25 %, respectively. The means were in normal range and the differences were statistically non-significant ($P > 0.05$) shown in Figure 4.18.

![Figure 4.18: Mean Corpuscular Hemoglobin Concentration (MCHC) (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicate level of significance ($P < 0.05$) among the groups.](image)

### 4.7 Biochemical Parameters:

Biochemical parameters were studied using standard kits and comparison of means between the groups for different biochemical parameters are shown as follows.

#### 4.7.1 Glucose:

Glucose, a fundamental nutrient required for normal brain function and other tissues, is under tight homeostatic control in order to allow for basic functioning of the animals. In ruminants, ingested carbohydrates are fermented to short-chain fatty acids by rumen microbes, and thus most glucose is synthesized by a process called as gluconeogenesis (Reynolds et al., 1988). As lactose is a major component in milk, gluconeogenesis is closely linked to lactogenesis (Mepham, 1993).

In the present study the mean glucose concentration of healthy (59.82 ± 1.81 mg/dL) animals was significantly ($P < 0.05$) higher than ketotic animals (sub-clinical and clinical). However, there was no significant ($P > 0.05$) difference in mean glucose concentration between sub-clinical ketotic (46.91 ± 5.61 mg/dL) and clinical ketotic (44.61 ± 2.64 mg/dL) groups (Figure 4.19). Our result is in accord with Elitok et al. (2010) who reported a significant decrease in serum glucose concentration of primary ketotic cows as compared to control cows. We observed a significant
negative correlation ($P = 0.003; r = -0.38$) between serum BHBA and glucose concentration.

![Glucose graph](image)

**Figure 4.19:** Glucose (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicate level of significance ($P < 0.05$) among the groups.

### 4.7.2 Total Protein:

The mean total protein in sera of healthy ($7.60 \pm 0.22$ g/dL), sub-clinical ketotic ($7.67 \pm 0.17$ g/dL) and clinical ketotic ($7.91 \pm 0.25$ g/dL) animals showed no significant ($P > 0.05$) difference (Figure 4.20).

![Total protein graph](image)

**Figure 4.20:** Total protein (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicate level of significance ($P < 0.05$) among the groups.

### 4.7.3 Albumin:

The recorded serum albumin concentration of healthy, sub-clinical and clinical ketotic animals was $2.64 \pm 0.04, 2.71 \pm 0.05$ and $2.80 \pm 0.09$ g/dL, respectively. The difference of means was non-significant ($P > 0.05$) among the groups (Figure 4.21).
Results and discussion

4.7.3 Globulin:

The mean globulin concentrations of healthy (5.05 ± 0.15 g/dL), sub-clinical ketotic (5.09 ± 0.37 g/dL) and clinical ketotic (5.11 ± 0.17 g/dL) animals showed a non-significant \((P > 0.05)\) difference (Figure 4.22).

![Globulin](image)

Figure 4.22: Globulin (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicates level of significance \((P < 0.05)\) among the groups.

4.7.4 A/G Ratio:

Mathematical calculation of albumin to globulin ratio for healthy, sub-clinical ketotic and clinical ketotic groups were 0.59 ± 0.01, 0.53 ± 0.03 and 0.53 ± 0.01, respectively. The means showed a non-significant \((P > 0.05)\) difference among the groups (Figure 4.23).

![A/G Ratio](image)
58

Figure 4.23: Albumin: Globulin ratio (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicates level of significance ($P < 0.05$) among the groups.

The plasma proteins are sensitive to nutritional influences but the changes are often subtle and difficult to detect and interpret. Dietary protein deficiency results in a decreased turnover of serum albumin. Immunoglobulins are affected only on severe protein restriction (Benditt et al., 1949), but the effects are reversible on protein repletion (Wissler et al., 1946). The stress causes a decrease in total protein, decrease in albumin and often an increase in globulin. High serum globulins are common in cattle with chronic infection (mastitis, cellulitis, liver abscesses, etc.) and high serum globulins will lower the A/G ratio (Russell and Russell, 2007). None of the cows sampled had evidence of chronic infection which would result in high serum globulins.

4.7.5 Non-esterified fatty acids (NEFA):

Non-esterified fatty acids (NEFA) are molecules released from triglycerides by the action of the enzyme lipase and are transported in the blood bound to albumin. They contribute only a small proportion of the body’s fat, however they provide a large part of the body’s energy. Measurement of NEFA is important in ketosis where negative energy balance results in the metabolism of fat. In response to decreased glucose availability during ketosis, an increase in lipolysis releases non-esterified fatty acids (NEFAs) which circulate throughout the body in the blood (Herdt, 2000). We also found a significantly ($P < 0.05$) higher level of serum NEFA concentration in clinical ketotic (1.09 ± 0.14 mM) animals as compared to sub-clinical ketotic (0.58 ± 0.01 mM) animals and healthy (0.40 ± 0.01 mM) animals (Figure 4.24). A similar finding was reported recently by Cao et al. (2017) that the serum NEFA concentration
of ketotic animals was significantly higher than the healthy animals. The correlation between serum BHBA and NEFA was highly significant ($P = 0.002; r = 0.377$).

![Non-esterified fatty acid](image)

Figure 4.24: Non-esterified fatty acid (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicate level of significance ($P < 0.05$) among the groups.

### 4.7.6 Triglycerides:

Triglycerides are the main constituents of body fat in humans and other animals. They are also present in the blood to enable the bidirectional transference of adipose fat and blood glucose from the liver. The mean triglyceride concentration of healthy, sub-clinical and clinical ketotic animals were 28.17 ± 1.88, 35.66 ± 2.99 and 50.60 ± 8.09, respectively. The triglyceride level was significantly ($P < 0.05$) increased in clinical ketotic group as compared to healthy group (Figure 4.25). However, the increase was non-significant between sub-clinical ketotic and healthy group. Also, the correlation ($r = 0.23$) between serum BHBA and triglycerides was positive but not significant ($P = 0.166$). Our result is in agreement with the reports of Nazifi et al. (2008) and Arya (2008), who observed highly significant ($P \leq 0.01$) increase in serum triglycerides level in ketotic cows. Similarly, Loor et al. (2007) also recorded increase serum triglyceride and decrease serum glucose level in induced ketotic dairy cattle. The rapid increase in the triglycerides during lactation may be due to increased demand of the udder for fatty acid synthesis for milk fat and to meet the energy demand in case of negative energy balance during early lactation (Tainturier et al., 1984).
Results and discussion

Figure 4.25: Triglycerides (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicates level of significance ($P < 0.05$) among the groups.

4.7.7 Cholesterol:

A significant ($P < 0.05$) reduction in cholesterol level was observed in sub-clinical ketotic (107.94 ± 18.97 mg/dL) and clinical ketotic (105.25 ± 31.54 mg/dL) animals as compared to healthy (155.66 ± 5.42 mg/dL) animals. However there was no significant ($P > 0.05$) difference between sub-clinical and clinical ketotic groups (Figure 4.26). A negative non-significant correlation ($P = 0.706; r = -0.48$) was observed between serum BHBA and cholesterol. The results of this study are similar to previous report of Ghanem and El-deeb (2010) in buffalo where they also found a significant decrease in serum cholesterol level in ketotic buffaloes compared to healthy. This decrease in cholesterol concentration could be attributed to the hampered liver function due to fatty liver condition which causes reduction in cholesterol formation (Grummer, 1995).

Figure 4.26: Cholesterol (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicates level of significance ($P <0.05$) among the groups.
4.7.8 High density Lipoprotein (HDL):

A significant \((P < 0.05)\) reduction in HDL concentration was recorded between healthy and ketotic animals (Figure 4.27). The mean HDL concentrations of healthy, sub-clinical ketotic animals and clinical ketotic animals were 54.30 ± 4.89, 25.90 ± 3.16 and 26.12 ± 4.52 mg/dL, respectively. The negative correlation \((r = -0.33)\) between serum BHBA and HDL was found significant \((P < 0.05)\). Our result coincides with the results of Marcos et al. (1990), Nasri and Baradaran (2004) and Turk et al. (2008). They also observed a significant decrease in HDL-cholesterol level in subclinical ketotic dairy cow in comparison to healthy groups.

![Figure 4.27: High density lipoprotein (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicates level of significance \((P <0.05)\) among the groups.](image)

4.7.9 Low density Lipoprotein (LDL):

Healthy \((41.52 ± 1.43 \text{ mg/dL})\), sub-clinical ketotic \((34.77 ± 6.04 \text{ mg/dL})\) and clinical ketotic \((35.41 ± 11.24 \text{ mg/dL})\) groups showed no significant \((P > 0.05)\) difference in mean LDL concentration (Figure 4.28).

![Figure 4.28: Low density lipoprotein](image)
Results and discussion

4.7.10 Aspartate transaminase (AST):

The mean AST activity of healthy, sub-clinical ketotic and clinical ketotic animals was 47.56 ± 3.16, 104.61 ± 27.58 and 124.19 ± 32.99 U/L, respectively. A significant \( (P < 0.05) \) increase in mean AST activity was observed in ketotic (sub-clinical and clinical) animals as compared to healthy animals. However, there was no significant \( (P > 0.05) \) difference in AST activity between sub-clinical and clinical ketotic groups (Figure 4.29). We found a significant positive correlation \( (P < 0.05; r = 0.36) \) between serum BHBA and AST. Similarly, Steen et al. (1997) also found greatly increased AST levels in ketotic cows compared with healthy ones. The infiltration of hepatic cells with fat increases cell membrane permeability with subsequent release of AST enzyme that serves as a good tool for metabolic diseases finding (Karasai and Schefar, 1984).

![Figure 4.29: Aspartate aminotransferase (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicates level of significance \( (P < 0.05) \) among the groups.](image)

4.7.11 Gamma- glutamyltransferase (GGT):

GGT plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione, drug and xenobiotic detoxification. The activity of this enzyme is relatively high in liver (Tenant, 1997) and its serum activity increases when hepatic tissues are damaged. The mean GGT activity of healthy, sub-clinical ketotic and clinical ketotic animals was 7.00 ± 0.72, 48.92 ± 16.44 and 112.32 ± 40.65 U/L, respectively. Many fold increase in GGT activity was recorded that was
significantly ($P < 0.05$) different among the groups (Figure 4.30). A highly significant positive correlation ($P < 0.001$; $r = 0.71$) was observed between serum BHBA and GGT levels. Our findings go in accord with Steen (2001) who also reported an increased serum GGT activity in cows with ketosis.

![Gamma glutamyl transferase](image)

Figure 4.30: Gamma glutamyl transferase (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicates level of significance ($P < 0.05$) among the groups.

4.7.12 Alanine transaminase (ALT):

A non-significant ($P > 0.05$) difference was observed in the mean ALT activity of healthy (23.22 ± 0.91 U/L), sub-clinical ketotic (28.74 ± 2.94 U/L) and clinical ketotic (25.70 ± 2.05 U/L) groups (Figure 4.31).

![Alanine aminotransferase](image)

Figure 4.31: Alanine aminotransferase (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicates level of significance ($P < 0.05$) among the groups.

4.7.13 Reduced Glutathione:

Reduced glutathione is a major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds as well as maintaining exogenous antioxidants such as vitamins C and E.
in their reduced (active) form (Scholz et al., 1989). The blood reduced glutathione concentration in healthy, sub-clinical ketotic and clinical ketotic animals was 7.46 ± 0.52, 10.08 ± 0.39 and 10.02 ± 0.32 mM. There was no significant ($P > 0.05$) difference in mean concentration of reduced glutathione among the groups (Figure 4.32).

![Reduced Glutathione](image)

Figure 4.32: Reduced Glutathione (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicates level of significance ($P < 0.05$) among the groups.

4.7.14 Catalase:

The mean catalase activity of erythrocyte hemolysate in healthy (2.89 ± 1.47 U/mL) animals was observed lower than ketotic animals. The mean activity of healthy animals was significantly ($P < 0.05$) lower than clinical ketotic (8.13 ± 0.14 U/mL) animals whereas it was non-significantly ($P > 0.05$) lower than the sub-clinical ketotic (5.59 ± 0.22 U/mL) animals (Figure 4.33). Our results do not agree with the report of Zhang et al. (2011) who also found a non significant difference in serum GSH-Px and catalase between the subclinical ketotic and healthy cows.

![Catalase](image)

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>SCK</th>
<th>CK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase (U/mL)</td>
<td>2.89</td>
<td>5.59</td>
<td>8.13</td>
</tr>
</tbody>
</table>

Our results agree with the report of Zhang et al. (2011) who also found a non significant difference in serum GSH-Px and catalase between the subclinical ketotic and healthy cows.
Figure 4.33: Catalase (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicate level of significance ($P < 0.05$) among the groups.

4.8 Ketone bodies:

Ketone bodies were qualitatively detected in urine and milk samples by Rothera’s test and Ross test respectively and compared with the detection of ketone body in urine by solid phase single reagent test. The urine ketone body concentration was estimated using aceatoacetate as standard with standardised qualitative modified nitroprusside test.

4.8.1 Solid Phase Single Reagent Test:

Qualitative detection of ketone bodies in urine using the developed solid phase single reagent test showed that the sensitivity of this test is 100% in all clinical ketotic and sub-clinical ketotic urine samples. Sensitivity of Rothera’s test in urine and Ross test in milk were 66.66 and 41.66 %, respectively.

Table 4.3: Comparative sensitivity of Rothera’s test, Ross test and Solid phase single reagent test to detect ketone bodies in urine or milk of sub-clinical ketotic animals.

<table>
<thead>
<tr>
<th>Name</th>
<th>Rothera’s test (Urine)</th>
<th>Ross test (Milk)</th>
<th>Solid phase single reagent test (Urine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mala</td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Kiran</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pandadi</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Vibha</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Sobhna</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ridhhi</td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Latika</td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Trushna</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Padmavati</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Ashwini</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Viveka</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Chandra</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Results and discussion

4.8.2 Quantitative Modified Nitroprusside Test

The mean ketone body concentrations of healthy, sub-clinical ketotic and clinical ketotic animals were 5.04 ± 0.46, 42.51 ± 4.89 and 74.29 ± 7.59 mg/dL, respectively. We observed a significant \((P < 0.05)\) difference in urine ketone body concentration among the groups (Figure 4.35). A highly significant positive correlation \((P < 0.001; r = 0.88)\) was recorded between serum BHBA and urine ketone body concentration.

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>SCK</th>
<th>CK</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/dL</td>
<td>5.04</td>
<td>42.51</td>
<td>74.29</td>
</tr>
</tbody>
</table>

Figure 4.35: Urine ketone bodies (Mean ± SE) of healthy, sub-clinical ketotic (SCK) and clinical ketotic (CK) groups. Small superscripts (alphabet) indicate level of significance \((P < 0.05)\) among the groups.