CHAPTER V
SUMMARY AND CONCLUSION

SUMMARY:
The Gir cows, in 3-5 weeks of lactation, were screened for serum BHBA concentration and were categorized into groups namely, (I) healthy (< 1.2 mM), (II) sub-clinical ketotic (1.2 - 3.0 mM) and (III) clinical ketotic (>3 mM). The present study showed that out of sixty animals, forty were found healthy (67 %), twelve sub-clinical ketotic (20 %) and eight clinical ketotic (13 %) animals. Animals in clinical ketotic groups were between 6-15 years of age and 2-7 parity. However, animals in sub-clinical group were between 4-12 years of age and 1-7 parity. 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. 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. A highly significant ($P = 0.001$) negative correlation ($r = -0.40$) between serum BHBA and BCS of the animals was found. 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. We also found a significantly ($P < 0.05$) positive correlation ($r = 0.49$) between serum BHBA and fat to protein ratio.

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. A highly significant ($P < 0.001$) positive correlation ($r = 0.78$) was found between BHBA and SCC.
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 the means were found in normal physiological range in all the groups.

Biochemical parameters were studied using standard kits and comparison of means between the groups was compared. 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 ketogenic (46.91 ± 5.61 mg/dL) and clinical ketogenic (44.61 ± 2.64 mg/dL) groups. A significant negative correlation ($P = 0.003; r = -0.38$) between serum BHBA and glucose concentration was observed.

The means of total protein, albumin, globulin and albumin to globulin ratio were found in normal physiological range for healthy, sub-clinical ketotic and there were no significant difference among the group.

The present study showed a significantly ($P < 0.05$) higher level of serum NEFA concentration in clinical ketogenic (1.09 ± 0.14 mM) animals as compared to sub-clinical ketogenic (0.58 ± 0.01 mM) animals and healthy (0.40 ± 0.01 mM) animals. The correlation between serum BHBA and NEFA was highly significant ($P = 0.002; r = 0.377$).

The mean triglyceride concentrations of healthy, sub-clinical and clinical ketogenic animals were 28.17 ± 1.88, 35.66 ± 2.99 and 50.60 ± 8.09 mg/dL, respectively. The triglyceride level was significantly ($P < 0.05$) increased in clinical ketogenic group as compared to healthy group. However, the increase was non-significant between sub-clinical ketogenic and healthy group. Also, the correlation ($r = 0.23$) between serum BHBA and triglycerides was positive but not significant ($P = 0.166$).

A significant ($P < 0.05$) reduction in cholesterol level was observed in sub-clinical ketogenic (107.94 ± 18.97 mg/dL) and clinical ketogenic (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 ketogenic groups.
A negative non-significant correlation ($P = 0.706; r = -0.48$) was observed between serum BHBA and cholesterol.

A significant ($P < 0.05$) reduction in HDL concentration was recorded between healthy and ketotic animals. The mean HDL concentrations of healthy, sub-clinical ketotic animals and clinical ketotic animals were $54.30 \pm 4.89$, $25.90 \pm 3.16$ and $26.12 \pm 4.52$ mg/dL, respectively. The negative correlation ($r = -0.33$) between serum BHBA and HDL was found significant ($P < 0.05$). However, healthy ($41.52 \pm 1.43$ mg/dL), sub-clinical ketotic ($34.77 \pm 6.04$ mg/dL) and clinical ketotic ($35.41 \pm 11.24$ mg/dL) groups showed no significant ($P > 0.05$) difference in mean LDL concentration.

The mean AST activity of healthy, sub-clinical ketotic and clinical ketotic animals was $47.56 \pm 3.16$, $104.61 \pm 27.58$ and $124.19 \pm 32.99$ 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. We found a significant positive correlation ($P < 0.05; r = 0.368$) between serum BHBA and AST. The mean GGT activity of healthy, sub-clinical ketotic and clinical ketotic animals was $7.00 \pm 0.72$, $48.92 \pm 16.44$ and $112.32 \pm 40.65$ U/L, respectively. Many fold increase in GGT activity was recorded that was significantly ($P < 0.05$) different among the groups. A highly significant positive correlation ($P < 0.001; r = 0.713$) was observed between serum BHBA and GGT levels. However, A non-significant ($P > 0.05$) difference was observed in the mean ALT activity of healthy ($23.22 \pm 0.91$ U/L), sub-clinical ketotic ($28.74 \pm 2.94$ U/L) and clinical ketotic ($25.70 \pm 2.05$ U/L) groups.

The blood reduced glutathione concentration in healthy, sub-clinical ketotic and clinical ketotic animals was $7.46 \pm 0.52$, $10.08 \pm 0.39$ and $10.02 \pm 0.32$ mM. There was no significant ($P > 0.05$) difference in mean concentration of reduced glutathione among the groups. Furthermore, the mean catalase activity of erythrocyte hemolysate in healthy ($2.89 \pm 1.47$ U/mL) animals was observed lower than ketotic animals. The mean activity of healthy animals was significantly ($P < 0.05$) lower than clinical ketotic ($8.13 \pm 0.14$ U/mL) animals whereas it was non-significantly ($P > 0.05$) lower than the sub-clinical ketotic ($5.59 \pm 0.22$ U/mL) animals.

Sensitivity of Rothera’s test in urine and Ross test in milk were 66.66 and 41.66 %, respectively for qualitative detection of sub-clinical ketosis samples.
However, it was 100% for developed solid phase single reagent test. All tests were equally sensitive for the detection of clinical ketosis. The mean ketone body concentrations, estimated by modified nitroprusside test, in urine samples 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. Also, a significant ($P < 0.05$) difference in urine ketone body concentration among the groups was recorded. A highly significant positive correlation ($P <0.001; r = 0.88$) was observed between serum BHBA and urine ketone body concentration.

**CONCLUSIONS:**

Based on serum BHBA concentration of Gir cows during 3-5 weeks of lactation, the incidence of sub-clinical and clinical ketosis is around 20 % and 13 %, respectively. Hematological parameters and serum protein profile is not affected and remain in normal physiological range in clinical and sub-clinical ketotic Gir cows. The body condition score, serum glucose, cholesterol and HDL levels decrease whereas AST, GGT, NEFA and triglycerides increase, significantly in ketotic cows compared to healthy cows. Somatic cell count and fat to protein ratio in the milk of Gir cows increase in sub-clinical and clinical ketosis. Solid phase single reagent test is more sensitive than Rothera’s test and Ross test for qualitative detection of ketone bodies. Quantitative modified Nitroprusside test is an economic and sensitive test to detect ketone bodies in urine capable of differentiating sub-clinical ketosis from clinical ketosis.