CHAPTER II
REVIEW OF LITERATURE

Since the early days of domestication, the cow have been gradually genetically transformed into today’s domestic cattle, through selecting those individuals that served people the best (Björnhag et al., 1989). This genetic transformation has pushed the limits of the cow’s metabolism, nutritional requirements and milk production. Along with genetic changes, changes in housing, feeding, and management is needed to meet the high production demands. In dairy cattle, breeding for an increased milk yield has led to pronounced energy deficit postpartum (Veerkamp and Koenen, 1999).

2.1 Negative Energy Balance and Ketosis

Cows experience a strongly negative energy balance in early lactation and are more prone to metabolic disorders (Collard et al., 2000). Negative energy balance (NEB) and suboptimal mineral levels are common in the transition period in cows (Goff, 2006), because of higher needs. The period from three weeks before to three weeks after calving is considered the transition period (Quiroz-Rocha et al., 2009). During this time, the cow leaves pregnancy and enters lactation, undergoing numerous physiological adaptations in the process. Ketosis is a common metabolic disorder related to negative energy balance in dairy cows and it occurs when adipose tissue is used to meet energy demands and non-esterified fatty acids (NEFA) are incompletely oxidized into ketone bodies resulting in ketosis.

Ketosis can be subclinical or clinical depending on the subjectivity of the clinical signs. Subclinical ketosis is defined as the presence of increased blood ketone concentrations without clinical signs. The definition of subclinical ketosis consists of a blood BHBA concentration greater than 1.2 mM (Ospina et al., 2010). Clinical ketosis has visible clinical symptoms and typically occurs within the first six to eight weeks post-calving, resulting in anorexia, licking and blindness, hard dry feces, rapid loss of condition and decreased milk production (Youssef et al., 2010).

Ketosis or acetonemia is one of the most prevalent metabolic disorders in high-producing dairy cows, showing the highest incidence within the first weeks of lactation (Asl et al., 2011). Symptoms in dairy cows affected by ketosis are inappetence, depressed general condition, and reduced milk production. Central nervous system symptoms are present in some cases, for example, muscle fibrillation,
incoordination, excessive licking or chewing, aggression or bellowing. Increased concentrations of both BHBA and NEFA are used as markers of SCK and are associated with an increased risk of developing various diseases, reproductive disorders, and changes in milk production (Suthar et al., 2013; Raboisson et al., 2014).

2.2 Body Condition Score

Body condition scoring is widely used in cattle to evaluate nutritional status and several scoring charts exist (Wildman et al., 1982; Edmonson et al., 1989; Gillund et al., 1999). 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 denotes an excessively fat cow and 3 is an average body condition.

Buckley et al. (2003) reported that only 30% of cows with a BCS of 3.25 at calving lost more than 0.5 units of BCS in early-lactation, while 50% of cows with a BCS of 3.5 at calving lost 0.5 units of BCS or more in early lactation. Therefore, the higher the BCS at calving, the more BCS will be lost in early-lactation. It is important to remember that this BCS loss means that the liver is presented with large quantities of nonesterified fatty acids for metabolism and the liver of the dairy cow is not capable of metabolizing large quantities of fat.

BCS, and change in BCS can thus be used to monitor nutrient supply, although BCS is subjective and retrospective, and thus not as useful in the early detection of health problems (Ingvartsen et al., 2003). From the dry period to early lactation, a physiological decline in body condition can be anticipated as a response to the use of body reserves. However, extensive decline in condition is negative for the individual and has been associated with increased incidence of ketosis (Kim and Suh, 2003).

Valde et al. (2007) found that cows in a fatter condition at calving lose more body weight and body condition over a longer period of time than cows in a thinner condition at calving. Cows with higher BCS are thus more prone to ketosis.
2.3 Hematological Parameters and Ketosis

Hematological parameters are very helpful for diagnosis of disease and disease conditions. However, there is no clear cut correlation of hematological parameters with sub-clinical or clinical ketosis. Many researchers have reported contradictory reports on relation of hematological parameters in ketosis condition.

Seker (1989) studied differential blood picture and reported eosinophilia in 30%, lymphocytosis in 27% and lymphopenia in 13% ketotic cattle. He also reported that as milk yield increase the hematocrit and hemoglobin values decrease.

Akhtar (1997) screened hematological parameters in blood sample of 15 Nili Ravi buffaloes with ketosis and compared them with healthy buffaloes. There was no significant change in erythrocyte, leukocyte count and PCV between healthy and diseased buffaloes. In diseased buffaloes relative neutrophil count was significantly higher and relative lymphocyte and eosinophil count were significantly lower.

Oppel et al. (2000) observed a close, significant retrospective correlation between glycated haemoglobin (GHb) and plasma glucose measured 4 and 2 weeks earlier, respectively in a study. It is suggested that metabolic disorders can be predicted earlier, and more accurately, than glucose measurement at calving, by sampling GHb earlier in pregnancy and possibly preventing ketosis.

Nazifi et al. (2008) stated that there were significant differences in packed cell volume (PCV) between the 25–30 days postpartum, the 55–60 days postpartum and the pregnant cows. The hematocrit level in the pregnant cows was significantly higher than that in postpartum cows ($P < 0.05$). The leukocyte count and hemoglobin concentration in the pregnant cows were significantly higher than that in postpartum cows in 25–30 days after parturition. The erythrocyte count in pregnant cows was significantly higher than that of postpartum cows in 55–60 days after parturition.

Quiroz-Rocha et al. (2009) observed in a study that total leukocytes and neutrophils were statistically different in both pre-calving and post-calving periods when comparing the cows in first lactation and cows in third or greater lactation groups, as well as between cows in second lactation and in third or greater lactation in the pre-calving period. Lymphocytes were significantly higher in cows in first lactation compared with cows in third or greater lactation in the pre-calving period. There were no significant differences in monocyte numbers. Eosinophils were statistically lower in both periods when comparing cows in first and second lactation.
Kumar et al. (2015) reported in the investigation which was conducted to evaluate the therapeutic efficacy of different drugs in twenty four (n=24) buffaloes suffering from primary ketosis (screened out of 145 buffaloes). Primary ketotic animals were diagnosed on the basis of clinical signs (selective anorexia, drastic reduction in milk yield and absence of any other concurrent diseases) and two positive urine tests (Rothera’s test and Keto-Diastix strip test). The mean blood values of eosinophil, TEC and MCV (p<0.05) differs significantly whereas the remaining blood parameters values were same before and after treatment the mean blood values of TLC, eosinophil, monocyte, TEC, MCV and MCHC (p<0.05) were significantly different in treated animals as compared to control . Likewise the mean blood values of Hb, TLC, neutrophil, eosinophil, monocyte, TEC and PCV (p<0.05) differs significantly.

Marutsova et al. (2015) stated that cows with clinical ketosis had higher hemoglobin (Hb) and hematocrit (HCT) values. Hematological analysis showed leukocytosis and lymphocytosis in cows with subclinical ketosis as compared to control group whereas clinical ketotic cows showed leucopenia. Body condition score (BCS) in cows with clinical ketosis was also found negatively correlated with higher blood BHBA concentrations.

Schulz et al. (2015) investigated in a study that animals in the higher body condition scores (HBC) group evidenced subclinical ketosis whereas lower body condition scores (LBC) animals were metabolically healthy. For in vitro examination with β-hydroxybutyrate (BHB) as a further stimulus, peripheral blood mononuclear cell (PBMC) counts of cows with and without subclinical ketosis (n = 5 per group) were observed. Counts of leucocytes, granulocytes and lymphocytes (LY) peaked at day 1 post-partum in HBC cows, with a more marked increase in heifers. In subclinical ketosis LY count increased again, with significantly higher values in the HBC group. The red blood cell (RBC) profile was affected by parity (counts were higher in heifers). PBMC from cows that were not pre-stressed with subclinical ketosis were more sensitive to increasing levels of BHB in vitro. Concentrations of BHB in vivo during subclinical ketosis did not alter the proliferative capability of bovine PBMC in vitro, which was first significantly decreased at a dosage of 5 mM BHB.
Gerspach *et al.* (2016) reported in a study of total of 50 cows in which left displacement of abomasum (LDA) was diagnosed in all cows and confirmed during laparotomy. Nine cows had no abnormal hepatic findings on histo-pathological assessment of the liver tissue. Fatty liver was diagnosed in 41 cows, including mild (*n* = 14), moderate (*n* = 22) and severe (*n* = 17) fatty liver. The number of days in milk was significantly higher in cows with normal livers. Hematocrit and hemoglobin concentrations were significantly lower in group 3 (severe) compared to group 2 (moderate).

Nightingale (2016) observed Hemoglobin concentrations and hematocrit percentages decreased on day 14 and were lower among Control cows from day -30 to 28. Neutrophil L-selectin surface protein concentrations decreased at parturition in Control cows in a study of seventy-five cows in their 1st to 8th lactation from one large commercial dairy in Eastern New Mexico. Whole blood was collected via jugular venipuncture from each animal at -7 ± 3 and -3 ± 3 days prior to their expected calving date based on the herd’s average gestation of 275 days carried calf. The same cow sampled prepartum was subsequently sampled at 7 ± 3 and 28 ± 3 DIM.

Singh *et al.* (2017) studied in of a six crossbred dairy cows in their early lactation period suffering from subclinical ketosis the mean Hb and PCV values showed a significant (*p*<0.05) decrease from far off dry period (FOD) period up to the fresh period, where as a non significant increase was noticed in the mean TEC and TLC levels from late pregnancy up to early lactation period, whereas post treatment the mean PCV levels showed a significant (*p*<0.05) increase and mean Hb and TEC levels showed a non-significant increase.

### 2.4 Biomarkers of Ketosis

Kulkarni *et al.* (1983) reported normal values of some biochemical constituents of blood in 6 crossbred lactating cows during the first three months of lactation. Mean values (±SE) and ranges of concentration were: Total proteins (g%) 6.53 ± 0.01 (6.41-6.63), Albumin (g%) 2.89 ± 0.29 (2.85-2.91), Globulin (g%) 3.46 ± 0.03 (2.96-3.68) and A:G ratio 0.80±0.10 (0.78-0.83).

Venkateswarlu (1993) observed increased serum AST levels (100.75 ± 6.50 IU/L) in cows suffering with subclinical ketosis.
Venkateshwarlu (1996) observed that the mean AST levels ranged between 32.08 ± 1.49 to 117.33 ± 16.58 IU/L in subclinical ketotic cows.

Whitakar et al. (1993) had chosen 1000 μmol/L of blood BHBA as a cutoff point to identify subclinical ketosis in groups of early lactating cows and reported that the blood BHBA concentrations for assessing subclinical ketosis ranged from 1000 μmol/L to 1400 μmol/L.

Anantwar and Bhoop Singh (1994) observed a significant decrease in blood glucose levels in subclinically ketotic buffaloes (55.32 mg/dl) while, the corresponding values for healthy animals were 62.84 mg/dl.

Balakishan (1994) reported increased AST levels before therapy as 106.87 ± 3.40, 123.12 ± 3.53 and 120.06 ± 2.95 IU/L respectively in group I, II and III subclinical ketotic buffaloes.

Mobilization of body fat is a normal process which enables the cow to augment the energy supply as the mobilized body fat avails energy for milk production to the cow. However, excessive mobilization of body fat elevates plasma non-esterified fatty acid (NEFA) concentration and increases its uptake by the liver, thereby leading to fatty liver syndrome and ketosis (Drackley, 1999).

Kennerman (1999) reported increase serum AST levels among animals suffering with subclinical ketosis.

The measured concentrations of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) for the success of adaptation to negative energy balance. NEFA reflects the magnitude of mobilization of fat from storage. BHBA indicates the completeness of oxidation (“Burning”) of fat in the liver. Ketone bodies (BHBA, acetone and acetoacetate) are intermediate metabolites of oxidation of fatty acids; as the supply of NEFA to the liver exceeds the ability of liver to completely oxidize the fatty acids to supply energy, the amount of ketone body production increases. Ketone bodies can be used by muscle as an alternative fuel source to glucose, sparing glucose for milk production (Herdt, 2000).

Glucose concentrations are under tight homeostatic control. Therefore, although glucose has a central role in metabolism, it is a poor analyte for monitoring or investigating herd problems. Glucose production in liver is controlled by the availability of substrates for glucose, the metabolic capacity of the primary cells of the liver (Hepatocyte) that synthesize glucose and hormonal status of the animal.
particularly insulin and glucagon. Controlled studies have demonstrated that fat infiltration in liver cells impairs their ability to synthesize glucose and to detoxify ammonia (Zhu et al., 2000).

Anjilappa (2001) recorded increased AST levels of subclinical ketotic buffaloes before therapy as 97.25 ± 4.51 U/L and reported that the decrease in AST levels post therapy was highly significant (P<0.01).

Ruminal and hindgut fermentation result in production of volatile fatty acids (VFA), with propionate being the major gluconeogenic precursor. Propionate contributes an estimated maximum of 32 to 73% to hepatic gluconeogenesis, whilst amino acids from dietary intake and skeletal protein mobilization contribute 10 to 30%. However, propionate supply during the transition period is limited (Drackley et al., 2001; Seal and Reynolds, 1993).

Sharma and Kumar (2001) analysed the blood and sera samples of 40 cows and buffaloes with subclinical ketosis and concluded that their corresponding values were similar in both but blood glucose was slightly higher in buffaloes.

The GGT, AST and albumin concentration were helpful for liver function test in cows with fatty liver (Sevinc et al., 2001).

Ingvartsen et al. (2003) observed that over-conditioned dry cows are more likely to suffer from ketosis and fatty liver, both of which may suppress immunity directly or through an excessive negative energy balance route. Glucose concentrations decrease at parturition and are lower in the first weeks of lactation than before calving or later in lactation.

Kim and Suh (2003) investigated the effect of body condition loss from the dry to near calving periods on the subsequent body condition change, the occurrence of postpartum diseases (including abomasal displacement, milk fever, ketosis). Cows were categorized based on body condition loss from the dry to near calving periods into two groups. The triglyceride and glucose concentrations did not differ from the dry period to month 4 of lactation between the two groups.

Sevinc et al. (2003) reported that mean GGT (U/L) values were significantly higher in cows with spontaneous ketosis (33.3 ± 4.5) than those of healthy cows (22.4 ± 2.4).

Sutkevicius and Cernauskas (2003) reported that hypoalbuminaemia was more frequent in cows during early postpartum periods.
Carrier et al. (2004) evaluate the performance of 3 cowside diagnostic tests for detection of subclinical ketosis, defined as a serum β-hydroxybutyrate (BHBA) concentration $\geq$1400 μ mol/L. On average, use of the Ketostix at the “small” cut-off point or the Keto test at 100 μ mol/L would result in no more than 3 or 4 false positives per 100 cows screened, with prevalence levels ranging from 5 to 30%, whereas the number of false negatives would range from one false negative at 5% prevalence to 7 or 8 false negatives at 30% prevalence. Finally, given their relative imprecision, use of any of these individual cow-side tests to estimate herd prevalence must be done cautiously, especially when only a small number of animals are sampled.

Nielsen and Ingvartsen (2004) reported propylene glycol (PG) increases insulin by 200–400% within 30 min after drenching, indicating that PG is absorbed rather quickly. Allocation of PG also increases plasma glucose, although the response is limited, probably because of the large increase in insulin. PG decreases plasma concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB), especially in early lactating cows with relatively high levels of NEFA. PG also reduces the triacylglycerol (TG) content of the liver and the concentrations of ketone bodies in milk and hence, has anti-ketogenic properties.

Oetzel (2004) studied 18 to 35% of cows have NEFA $>$0.4 mmol/L in the last week before calving. He suggested an “alarm” threshold of 10% prevalence of subclinical ketosis, based on serum BHB. Prevalence of subclinical ketosis was found around 15% studied in Canada in comparison to average prevalence of 20%. Adjusting for cow-side test performance, a threshold of 10% true prevalence of subclinical ketosis corresponds to an apparent prevalence (proportion of tests that are positive) of 25% when using the Keto-Test with a 100 μ mol/L cut-point, or 11% at the 200 μ mol/L cut-point.

Osborne (2004) evaluated 136 transition cows, 24 had BHBA concentrations $\geq$ 1400 μ mol/L of serum in the first week post-calving (17.6%). There was a significant association between NEFA concentration in the week prior to calving and BHBA concentration in the first week post-calving. A nearly 5-fold increased risk of subclinical ketosis was noted when the NEFA concentrations in the week before calving were greater than 0.7 mmol/L ($OR$=4.8, $P=0.04$).
Van Saun (2004) reported in a study to establish diagnostic relationship between pre-partum blood metabolite concentrations and post-partum health status, revealed that all the sick cows had lower albumin, glucose and cholesterol and higher AST, BHBA and NEFA compared to healthy cows in the fresh period. Cows, with close up period albumin concentrations < 3.25 g/dl and fresh period < 3.30 g/dl were 1.46 and 1.79 times more likely to experience a disease event. Within fresh cows, cholesterol concentrations increased with increasing albumin concentrations. Similarly, the cows in which NEFA values were >0.4 mEq/L in either close up or fresh period samples, were 1.57 and 1.47 times more likely to have a disease event, respectively and the disease risk was greater if NEFA concentration was >0.6 mEq/L at close up and fresh period. He also reported that B-hydroxybutyrate (BHB) concentration is most commonly used for ketosis. Concentrations of BHB < 26 mg/dL and > 14.5 mg/dL represent animals with subclinical ketosis. The concentrations ≥ 26 mg/dL are defined with clinical ketosis. Prior to calving, BHB concentrations generally do not exceed 6-8 mg/dL, unless the animal is in negative energy balance or consuming ketogenic silage. Following calving, BHB concentrations can become greatly elevated. Cows with BHB concentrations above 10 or 14 mg/dL are 3.2 and 4.3 times at greater risk for postpartum disease.

The ketone bodies i.e., BHB, acetoacetate, and acetate, are formed by incomplete oxidation of NEFA in the liver. Another source of BHB in blood is ruminal butyrate that is oxidized to BHB in the rumen wall. Ketogenesis is part of the normal energy metabolism in ruminants. If glucose concentrations are low, more ketone bodies are produced in the liver to meet the energy needs of body tissues. Concentrations of BHB increase post partum and peak 2–4 weeks post partum (Cavestany et al., 2005; Ingvartsen et al., 2003).

Dann et al. (2005) determined the effect of pre partum intake, postpartum induction of ketosis, and periparturient disorders on metabolic status in dairy cows whereby they found that pre partum intake did not affect postpartum metabolic status or milk yield. However cows in which ketosis was induced, had lower intake, milk yield, and serum glucose concentration but higher concentrations of non esterified fatty acids and β-hydroxybutyrate in serum and total lipid and triacylglycerol in liver in comparison to control cows.
Kokkonen et al. (2005) reported increased level of NEFA in lactating cows as compared to non-lactating cows. Plasma NEFA concentrations were in the range of 100 to 2000 μEq/Litre in cows and were low in low producing cows.

The increase in NEFA is part of the physiological response necessary to meet higher energy demands at the onset of lactation, but excess NEFA concentrations are detrimental; for example, high NEFA concentration is toxic to peripheral tissue and negative affects fertility (Adewuyi et al., 2005; Leroy et al., 2005).

Stojevic et al. (2005) examined the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) in the plasma of 120 dairy HF cows. Animals were divided in 4 groups. The first, second, third and fourth group consisted of animals from the 10th - 45th, 46th- 90th, 91st day of lactation until the end of milk production. Cows in the dry period comprised the fourth group. The highest activity of AST was determined in the first production period, while enzyme activities in the second and third periods were higher than in the dry period. ALT activity showed a statistically significant increase from the 46th day of lactation until the dry period, and activity in the second and third periods were statistically higher than in the dry period. GGT activity in the first production period and in the dry period was statistically higher in comparison with the second and third periods.

LeBlanc et al. (2006) reported elevated pre-fresh non-esterified fatty acids (NEFA) concentration (≥ 0.4 mmol/L) and post fresh β-hydroxybutyrate (BHB) concentration (≥1200-1400 μmol/L) are recognized risk factors for ketosis and left displaced abomasums.

Blood concentrations of NEFA, beta-hydroxybutyrate (BHB), glucose and cholesterol have been associated with energy metabolism in dairy cows and may be used as indicators of NEB (Macrae et al., 2006; Oetzel, 2004; Agenas et al., 2003; Ingvartsen et al., 2003; Kim and Suh, 2003; Kronfeld, 1972).

Mordak and Nikpon (2006) compared the values of selected blood parameters in 30 clinical healthy cows in periparturient period that did not develop any pathological signs of disturbance just before, during or after calving. They found significant differences in the value of SGOT before and after calving. However, large differences were observed in the values of total proteins and glucose without any significant difference.
Kanna (2007) reported glucose levels were significantly (P<0.1) higher in high BCS than medium BCS cows (54.03 ± 3.02 vs 43.88 ± 2.33 mg/dL).

Radostits *et al.* (2007) mentioned a mini metabolic profile test which measures levels of blood glucose and albumin in cows between 4 and 10 weeks after calving that can serve as a sufficient test to assess the adequacy of energy and protein intake. The samplings were done at intervals of 4-6 weeks. The time of sampling can affect the results. The values will change with the season and stage of lactation.

Sakha (2007) stated that blood glucose levels (mg/dl) in cows with subclinical ketosis were significantly lower (30.63 ± 1.44) than in cows without subclinical ketosis (43.6 ± 0.72).

Cholesterol is secreted into the blood stream as VLDL, and low concentrations of cholesterol may impair the transport of TAG from the liver. Cholesterol concentrations are low at calving and increase slowly over the first weeks after calving (Van Knegsel *et al.*, 2007; Cavestany *et al.*, 2005).

The metabolic diseases of the dairy cows are manifestation of the cow’s inability to cope with the metabolic demands of high production and they continue to be a cause of economic loss to the dairy industry and an animal welfare concern (Mulligan and Doherty, 2008).

Nazifi *et al.* (2008) stated that there was significant (p< 0.05) increase in serum GGT levels to 8.93 ± 1.0 U/L in subclinical ketotic cows when compared to healthy cows (7.33 ± 0.73 U/L) and also studied 77 cows within two months of lactation and stated that cows with serum concentration of BHBA > 1000 µmol/lit were considered sub-clinically ketotic.

Stengarde *et al.* (2008) conducted metabolic profiling in five high-producing Swedish dairy herds having history of abomasal displacement and ketosis and found that all herds had over conditioned dry cows that lose body condition substantially during the first 4–6 weeks postpartum, two herds had elevated levels of NEFA pre-partum and three herds had elevated levels postpartum, while one herd had low levels of cholesterol postpartum.

Djokovic *et al.* (2009) reported that glucose levels were significantly lower (P<0.01) in the puerperal cows than in the cows examined during the maximum lactation period (90-100 days), which suggested a decreased gluconeogenesis in the liver. Significantly lower blood levels of total protein (P<0.01), albumin (P<0.01),
and triglyceride (P< 0.05) were recorded in the puerperal cows, which suggested the reduced synthetic capacity of liver cells during early lactation in cows. Significantly increased (P<0.01) AST and GGT activities in the blood in the puerperal cows suggested the disturbed morphological and functional integrity of liver cells and the release of these intracellular enzymes into the blood.

Duffied et al. (2009) reported subclinical ketosis (BHB > 1200 to 1400 μmol/L) in the first or second week after calving is associated with 3 to 8 times increased risk of left displacement of abomasums, 3 times greater risk of metritis when serum BHB in week 1 was > 1200 μmol/lit, 4 to 6 times increased risk of clinical ketosis increased probability of subclinical endo-metritis at week 4 postpartum and increased duration and severity of mastitis but not with the incidence of mastitis. Milk yield at first test was reduced by 1.9 kgs /day when BHB was >1400 μmol/L in week 1 and by 3.3 kgs /day when BHB was >2000 μmol/lit in week 2. Cows with serum BHB >1800 μmol/L in week 1 had >300 kgs lower projected production for the whole lactation.

Hammon et al. (2009) studied the elevated liver fat content in high-yielding dairy cows during the transition period. They observed that high fat mobilization, high liver fat content, and severe NEB in high-yielding dairy cows may not always be a consequence of impaired farm management, but might be a consequence of individual cow factors that cannot be easily alleviated by the farmer when using feeding management. Individual cow factors were responsible for differences in energy metabolism during the transition period.

Iwersen et al. (2009) determined the diagnostic performance of an electronic β-hydroxybutyrate (BHBA) hand-held meter (Precision Xtra) for ketosis in dairy cattle. Specific objectives were to compare the electronic BHBA meter with serum BHBA concentrations determined photometrically and 2 commonly used chemical cow side tests (Ketostix and Ketolac) and to evaluate accuracy. The Precision Xtra test is a useful cow-side ketone test for detection of subclinical ketosis in postpartum dairy cows. Using whole blood and a cut off value of ≥1,400 μmol of BHBA/L of blood, the Precision Xtra test achieved excellent test characteristics and a higher diagnostic performance than 2 chemical dipsticks.

Quiroz-Rocha et al. (2009) reported in a study that all biochemical analytes (β-hydroxybutyrate, fatty acids, glucose and cholesterol) were statistically different
between pre-calving and post-calving groups. Through the examination of groups by lactation, BHB showed a significantly lower mean at cows in second lactation compared to cows in third or greater lactation in the post-calving period. Fatty acid concentrations were statistically different in all groups pre-calving and post-calving except between first lactation and third lactation in post-parturient cows. Cholesterol was significantly lower in second lactation compared with third lactation in the pre-calving period, but not between other comparisons. Glucose concentrations were different except between second lactation and third or greater lactation in the pre-calving period when reference limits for the weeks before and after calving were determined in dairy cows of the animals that had adverse clinical outcomes after calving.

Djokovic et al. (2010) reported significantly lower (P<0.05) blood levels of triglyceride, total protein and albumin in the puerperal cows, which suggested the decreased synthetic capacity of liver cells.

Duffield (2010) revealed that elevated pre fresh non-esterified fatty acids (NEFA) concentration (≥ 0.4 mEq/L) and post fresh β-hydroxybutyrate (BHB) concentration (≥14.4 mg/dL) were recognized risk factors for ketosis and left displacement of abomasum.

Elitok et al. (2010) conducted studies on primary ketosis in cows and recorded an increase (P<0.05) in gamma-glutamyl transferase.

Forslund et al. (2010) reported that dairy cows with ketosis (BHBA > 1.5 mmol/L) have a normal concentration of glucose in blood with ketonemia.

Serum cholesterol concentration was significantly decreased in cattle with moderate and severe fatty liver compared to the healthy cows and cows with mild fatty liver. The serum cholesterol concentration was inversely related to NEFA concentrations means decreased serum cholesterol, higher NEFA and higher NEFA/cholesterol ratio were recorded in cows with fatty liver (Kalaitzakis et al., 2010; Holtenius, 1989).

Hubbard et al. (2010) reported that the gold standard diagnostic test for SCK is plasma or serum concentration of β-hydroxybutyric acid (BHBA). Using this test, a threshold of 1.4 mmol/L has been found to be the most accurate for detecting cows with SCK.
LeBlance (2010) observed high NEFA (> 0.4 mmol/L) in the last 7 to 10 days before expected calving is associated with increased risk of displaced abomasum, retained placenta, culling before 60 days in milk, and less milk production in the first 4 months of lactation. Subclinical ketosis (Serum BHB > 1200 to 1400 μmol/L) in the first or second week after calving is associated with increased risk of DA, metritis, clinical ketosis, endometritis, prolonged postpartum anovulation, increased severity of mastitis, and lower milk production in early lactation. There are several validated and practical tools for cow-side measurement of ketosis.

Ospina et al. (2010) elevated concentrations of NEFA and BHBA in the transition period predicted clinical disease in dairy cow. The standard value of NEFA concentrations is ≥0.3 mEq/L for cattle, 14 to 2 day pre partum and NEFA concentrations ≥0.6 mEq/L and BHBA ≥10 mg/dL for cattle 3 to 14 day postpartum. Both pre- and postpartum NEFA concentrations and BHBA concentrations above these critical thresholds were associated with increased risk for subsequent disease (e.g., Displace abomasum, Clinical ketosis, metritis or Retained placenta).

Ketosis commonly results either from the lack of sufficient glucose precursors available for energy production or from a reduced gluconeogenic capacity by the liver and it is characterized by elevated concentrations of the ketone bodies acetoacetate, acetone and β-hydroxybutyrate in the blood, milk and urine. Serum β-hydroxybutyrate concentrations are typically dichotomized to distinguish between normal and hyper ketonemic cattle, with frequently recommended cut points of 1.000 to 1.400 μmol/L (Rollin et al., 2010).

Sahinduran et al. (2010) observed significant increased BHBA concentration in the blood and serum activity of AST, ALT and GGT in ketotic cows. However, the concentration of glucose was significantly lower in these animals as compared to control.

Sravanthi et al. (2010) recorded low levels of serum albumin and serum total proteins in subclinical ketotic crossbred cows.

Stengarde (2010) studied the cows with displacement of abomasum displayed blood profiles indicating a severely altered energy metabolism (NEFA, BHB, cholesterol), liver cell damage (AST) and inflammatory responses (haptoglobin). At the herd level, energy markers (NEFA, glucose, cholesterol) indicated altered metabolism in cows in high-incidence herds compared with cows in low-incidence
herds. The markers of liver cell damage and inflammation were not different between high and low-incidence herds. Among high-incidence herds, BCS and change in BCS, and one metabolic marker (NEFA) were found most useful to pinpoint herd problems. Large herd size, high individual milk production level, keeping all dry cows in one group, and not cleaning the feeding platform daily, were found to be risk factors for a high incidence of displaced abomasum or ketosis at the herd level.

In a study, Tripathi et al. (2010) observed that serum total cholesterol, HDL cholesterol, triglycerides, phospholipids increased from early to mid stage of lactation and then decreased from mid to late stage of lactation, while LDL cholesterol increased with advancing stage of lactation. Serum NEFA differs significantly with decreased level during mid lactation. The correlation between serum lipid profile with milk fat per cent was non-significant during all stages of lactation.

Asl et al. (2011) determine the cut off point for NEFA and glucose concentrations for diagnosis of sub clinical ketosis, found that the optimal cut off point of $>0.26$ mmol/L for NEFA and $<2.26$ mmol/L for glucose with corresponding 82.54 per cent sensitivity and 91.89 per cent specificity for NEFA and 44.44 per cent sensitivity and 78.38 per cent specificity for glucose.

Cozzi et al. (2011) measured concentrations of a variety of blood-based markers in 740 Holstein cows in 33 dairy herds. They reported significant herd variance components for albumin as well as parity and season of production effects on total protein and globulin; however detailed study of diets or management practices was not conducted in their survey.

The values of AST were statistically higher ($P<0.05$) in early lactation in cows than in late pregnant cows and no significant difference ($P>0.05$) was observed between GGT activities in the two groups. Given that AST activity higher than 100 U/L is indicative of hepatic lesions, 2 early lactation cows (13.3%) in the study suffered from some degree of hepatic lesions, probably due to fat infiltration. These animals included 2 out of 7 cows considered to be ketotic and had blood NEFA values above 0.70 mmol/L. Meanwhile, none of the late pregnant cows had AST values higher than 100 U/L. A positive correlation between AST activity and lipomobilization (NEFA values) was observed by the significance coefficient ($r = 0.34$, $P<0.05$). In this study, the data regarding liver enzymes suggested that the process of
lipomobilization was sufficient to cause liver lesions in 13.3% of the early lactating cows (Gonzalez et al., 2011).

Praveena (2011) reported decreased mean serum total protein levels as 5.80 ± 0.05 g/dl in buffaloes which were positive for urinary ketones.

Piccione et al. (2011) measured serum protein fractions in cows during the late pre-partum and early post-partum periods and determined that serum total protein concentrations decreased from the pre-partum period to wk 1 post-partum, with decreased concentrations of globulins largely responsible for the decline in total protein concentrations.

Rezaeisaber et al. (2011) evaluated fatty liver syndrome in dairy cattle in Tabriz by measurement of NEFA and triglycerides serum values. Triglycerides aggregation in liver in last month of pregnancy had occupied more than 5% liver cells and amount of NEFA was more than 900 meq/Lit, being nonspecific and some other reasons. The results showed that NEFA had a direct relationship with TG. Thus, with elevation of NEFA serum values, TG also increases.

Sharif et al. (2011) reported mean level of BHBA and glucose in ketotic and non ketotic cows as 2.7 ± 0.8μmol/lit, 0.637 ± 0.2μmol/lit and 30.72 ± 1.47 mg/dl, 43.9 ± 0.8mg/dl respectively.

Xu et al. (2011) reported in a study in which the results of CPE-I (CPT-I) mRNA expression in cultured bovine hepatocytes using real-time reverse transcription polymerase chain reaction and ELISA methods. CPT-I transcription increased translating and translating increased with 0 to 3.0 mmol / L ( P <0.01) CPT-I transcription and translation was raised significantly from when the NEFA concentrations increased from 0 to 1.2 mmol / L, when significantly more when the NEFA concentrations increased from 1.2 to 4.8 mmol / L ( P<0.01) A high concentration NEFA was found to reduce fatty acid oxidation, potentially explaining the development from NEB to ketosis in dairy cows.

Zhang et al. (2011b) measured serum concentrations of sodium, potassium, magnesium and iron in dairy cows with subclinical ketosis and compared with healthy cows. The subclinically ketotic cows had significantly higher levels of non-esterified fatty acids and β-hydroxybutirate in serum and significantly lower levels of blood glucose ( p < 0.01) but no significant differences were observed, suggesting that the mineral elements measured are not involved in the pathogenesis of subclinical ketosis.
Liu et al. (2012) reported the effects of post partum enzymes metabolic status in Holstein cows on 1 week pre partum (week 1), days delivery (week 0) and 1-9 weeks postpartum (week 1-9). They were analyzed for Aspartate aminotransferase (AST), Alanine Aminotransferase (ALT), Gamma-Glutamyltransferase (GGT) and its activities. The results showed a higher activity of AST in cows during the 1-3 weeks than others. ALT activity indicated a statistically significant increase from the 5-7 weeks of lactation and activity in the 7th week postpartum periods significantly reached to the peak. GGT activity in the ante partum 1 week until delivery day was significantly lower in comparison to the first to reach the 9th weeks postpartum. Therefore, the activities of AST, ALT and GGT could be significantly changed in the blood plasma of Holstein cows.

Saber et al. (2012) measured NEFA serum values in fatty liver syndrome. The results showed the elevation of NEFA serum values.

Zhang et al. (2012) observed that ketosis is a common metabolic disorder frequently in dairy cows during the early lactation period. It is characterized by increased levels of ketone bodies in the blood, urine, and milk. Subclinical ketosis (SCK) in dairy cattle is an excess level of circulating ketone bodies in the absence of clinical signs of ketosis. Usually, detection of SCK is carried out by testing the ketone concentrations in blood, urine, and milk. The Ketolac BHBA test strip (with a cut-off threshold of 200 mM of BHBA in milk) is potentially useful tools for the routine monitoring of SCK in early postpartum dairy cows.

Djokovic et al. (2013) reported that cows in early lactation had significantly higher levels of serum BHB and NEFA, and lower glycaemia compared to the late pregnant cows. High lipomobilization (NEFA>0.4 mmol/L) was detected in 6 (40%) of early lactation cows but in none of the late pregnant cows, while subclinical ketosis (BHB>1.2 mmol/L) was detected in 14 (94.4%) of the early lactation cows and 4 (26.6%) of the late pregnant cows. AST activities above 100 U/L were detected in 2 early lactation cows and in none of the late pregnant cows. TG levels below 0.12 mmol/L and glucose below 2.5 mmol/L were found in 7 (44%) and 10 (66.6%) of the early lactation cows, respectively, and in none of the late pregnant cows. Early lactation cows were found to have lower blood serum levels of TG and albumin activities and higher concentrations of TP and AST activities compared to the late pregnant cows. The results of blood serum levels of glucose, TG, BHB, NEFA and
AST in early lactation cows suggest metabolic disorders associated with ketosis, and some degree of hepatic lesions, probably due to fat infiltration.

Ilic et al. (2013) evaluated the metabolic status of early and mid-lactation in dairy cows through changes in blood biochemical indicators. Blood samples were collected to measure beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), triglycerides (TG), glucose and the activity of aspartate transaminase (AST). Early lactation cows had significantly higher (P<0.05) values of blood BHB and NEFA, and lower glycemia (P<0.05) and TG (P>0.05) values compared to mid lactation cows. High lipomobilization (NEFA > 0.4 mmol/L) and subclinical ketosis (BHB > 1.2 mmol/L) were detected in 6 (40%) and 14 (94.4%) early lactation cows, respectively, and in none of the mid lactation cows. AST activities above 100 IU/L were detected in 2 early lactation cows and none of the mid lactation cows. TG concentrations below 0.12 mmol/L were found in 7 (44%) early lactation cows and 2 (13.3%) mid lactation cows. Glucose levels were below 2.5 mmol/L in 10 (66.6%) early lactation cows and 5 (33.3%) mid lactation cows. Blood serum values for glucose, TG, BHB, NEFA and AST showed that early lactation cows suffered from metabolic disturbances, which were associated with ketosis, and some degree of hepatic lesions, probably due to fat infiltration. They suggested that NEFA was useful indicators of the metabolic status of dairy cows during lactation.

Padmaja and Rao (2013) reported high levels of AST (138.5 ± 13.35U/L) in subclinical ketotic buffalo cows compared to the normal levels of AST (113.70 ± 4.46U/L) in healthy animals and also increased mean levels of GGT (56 ± 5.26U/L) in sub clinical ketotic buffalo cows when compared to levels of GGT (37.13 ± 2.94U/L) in normal buffalo cow. They also reported low levels of serum albumin and serum total proteins before therapy in subclinical ketotic buffalo cow when compared to healthy animals.

Rezaeisaber et al. (2013) measured the NEFA and BHBA serum values in early lactation in hepatic lipidosis. In this inspection, age, body condition score and Pregnancy status of animals was investigated. At the time of testing, sera were defrosted and NEFA levels in serum by Randox kit and Auto analyzer were measured. In this study, levels of BHBA were measured by Pars test kits and by spectrophotometric method. Results showed that based on Pearson’s Correlation index there is a direct correlation between NEFA and BHBA serum values. This index
indicates a direct effect of NEFA on BHBA serum values. Thus, with elevating of NEFA serum values, the BHBA values also increased.

Suthar et al. (2013) reported the prevalence of SCK and its relationships with postpartum metritis, clinical ketosis, displaced abomasums and mastitis in European dairy farms. He found that the overall prevalence of SCK was 21.8 % ranging from 11.2 to 36.6 %. Furthermore, the cows with SCK had 1.5, 9.5 and 5.0 times greater odds of developing metritis, clinical ketosis and displaced abomasums, respectively.

Wilson and Goodell (2013) measured Beta-hydroxybutyrate (BHB) in blood or milk of dairy cattle after calving for detection of ketosis. Results show that the BHB test methods agreed well for most non-ketotic cows, but tests did not agree well on classification of ketotic cows. Calibration improvements are a priority for improved Fossomatic testing of BHB in milk.

Compton et al. (2014) studied the prevalence of subclinical ketosis in pasture grazed dairy cows and stated that blood BHBA concentration was ≥1.4 mmol/lit in subclinical ketosis affected cows.

Schulz et al. (2014) stated that the subclinical ketotic cows had significantly higher values of non-esterified fatty acids (NEFA) and AST levels in serum.

Sun et al. (2014) reported mean plasma glucose values as 1.90 ± 0.7, 2.70 ± 0.63 and 3.37 ± 0.58 mmol/lit in clinical ketosis, subclinical ketosis and control group respectively in 7-21 day post calving Holstein cows.

Kumar et al. (2015) reported in a study in which the disease was confirmed in 24 buffaloes as primary ketosis on the basis of clinical signs (selective anorexia, drastic reduction in milk yield), absence of any other concurrent disease and two urine tests. Comparison of infected was made with eight apparently healthy buffaloes kept as control. Biochemical findings show hypoglycemia, hypoproteinemia hypercholesterolemia and high triglycerides activity in affected animals as compared to control group.

Marutsova et al. (2015) reported the results of blood β-hydroxybutyrate concentrations and BCS in all cows included in experiment. Cows from group (SCK) and group (CK) BHBA levels were higher –1.57±0.55 mmol/L (p<0.05) and 4.75±1.36 mmol/L (p<0.001) respectively, compared to cows from the control group 0.30±0.16 mmol/L and average BCS in control animals was 3.55±0.36, in SCK cows 3.25±0.27 and in cows with clinical ketosis group 2.51±0.31.
Sun et al. (2015) reported a significant correlations between BHBA and glucose (R = -0.474), BHBA and NEFA (R = 0.520) or AST (R = 0.525) in high producing cows during early lactation.

Gerspach et al. (2016) observed the activity of the AST was significantly higher in cows with fatty liver compared to cows with normal livers in a study of a total of 50 cows. LDA was diagnosed in all cows and confirmed during laparotomy. Nine cows had no abnormal hepatic findings on histopathological assessment of the liver tissue. Fatty liver was diagnosed in 41 cows, including mild (n = 14), moderate (n = 22) and severe (n = 17) fatty liver.

Kachhawaha et al. (2016) reported a significantly increased serum Glucose level with decreased in NEFA and BHBA levels following treatment of subclinical ketosis with propylene glycol and choline bicarbonate in crossbred dairy cows.

Akgül et al. (2017) reported in a study when subclinical Ketosis group compared to control group, BHBA levels were higher (p<0.001), while serum glucose levels were lower (p<0.001). On the other hand, albumin, GGT levels did not differ between study groups.

Alekish et al. (2017) reported in the investigation using 72 *N. caninum* seropositive cows and 61 seronegative dairy cows (control). Serum from all cows was tested to determine their *N. caninum* status (seropositive vs seronegative) using commercially available indirect enzyme-linked immunosorbent assay test kit (iELISA). Student independent t-test showed that there was a significant difference in the serum concentrations of BHB, AST and ALT between seropositive and seronegative cows. Serum concentrations of BHB, AST, and ALT were significantly elevated in seropositive cows compared to their values in seronegative cows. Results of this study indicate a possible relationship between *N. caninum* seropositivity and certain metabolic diseases such as ketosis and fatty liver syndrome in dairy cows.

Cao et al. (2017) observed in an experiment with a cut-off point of β-hydroxybutyrate (BHBA) was 1.20 mmol/L based on that 21 control and 17 ketotic Holstein Friesian cows selected. In the ketosis group, concentration of glucose (GLU) was decreased, and aspartate aminotransferase (AST) activity as well as BHBA and non-esterified fatty acid (NEFA) contents were increased.
Daros et al. (2017) reported that the overall prevalence of SCK, metritis and incidence risk of retained placenta in grazing dairy herds in Brazil were 21, 11 and 14%, respectively.

El-Deeb and Bahr (2017a) observed in an experiment that there was a significant (p≤0.05) increase in the levels of aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), non-esterified free fatty acids (NEFA) and β-hydroxybutyric acids (BHBA) in dairy cows affected with ketosis compared to control. Conversely, a significant (p≤0.05) decrease in the levels of glucose, total cholesterol, cholesterol ester, free cholesterol and triacylglycerol (TAG) were detected in diseased cows compared to control.

Ghanem et al. (2017) reported in a study where ketosis was diagnosed in 10 lactating cows in private farm in Qaluabia government based on the blood level of Non-esterified fatty acid (NEFA) and Beta hydroxy butyric acid (BHBA). Another 10 healthy cows with normal NEFA and BHBA were used as control. Clinically, the affected cows had partial or complete anorexia with reduction of rumen movement. Biochemically, there was a significant (P< 0.05) increase in AST, ALT in addition to NEFA and BHBA in ketotic group compared to control. On the other hand, there was significant decrease (P < 0.05) in triacylglyceride and albumin.

Man et al. (2017) stated in Multiparous Holstein cows between 3 to 9 d in milk were screened for hyperketonemia using a handheld meter 3 times per week, and enrolled at whole blood BHB concentration ≥1.2 mmol/L to 1 of 4 treatment groups: (1) 500 mL of a 50% dextrose solution i.v. once daily for 3 d (GLU, n = 9), (2) 300 mL of propylene glycol as a drench once daily for 3 d (PG, n = 9), (3) a combination treatment of a 500 mL of 50% dextrose solution i.v. and 300 mL of propylene glycol orally once daily for 3 d (GLU+PG, n = 8) and (4) an untreated control group (control, n = 8). Blood samples were collected immediately before as well as at 1, 2, 4, 8, 12, 24, 36, 48, 60, and 72 h after administration of the first treatment in which overall least squares means (95% CI) of whole blood BHB concentrations between 1 h and d 11 relative to first treatment were 1.11 (0.95 to 1.30), 1.26 (1.07 to 1.47), 0.96 (0.81 to 1.13), and 1.53 (1.30 to 1.80) mmol/L for the GLU, PG, GLU+PG, and control groups, respectively. Treatment with both glucose and propylene glycol led to a greater magnitude and more prolonged decrease in BHB concentrations compared with individual treatments. The NEFA and glucagon concentrations were lower
immediately after treatment in GLU and GLU+PG groups compared with control, and treatment with both glucose and propylene glycol was associated with a greater increase in glucose and insulin concentrations immediately after treatment compared with control and GLU treatment alone.

Singh et al. (2017) reported in the study conducted to check the efficacy of Propylene glycol (PG) which was given @ 200 ml per day orally for 5 days in the treatment of subclinical ketosis and to study its effect on various metabolic parameters. A significant decrease was noted in the mean plasma Beta Hydroxyl Butyric Acid (BHBA) and Non Esterified Fatty Acid (NEFA) values, along with a significant increase in the mean plasma glucose and total plasma proteins levels after treatment.

Van der Kolk et al. (2017) stated that in mammals, excess energy is stored primarily as triglycerides, which are mobilized when energy demands arise and cannot be covered by feed intake. There is general agreement that fatty acid β-oxidation capability is limited in the liver of (ketotic) cows. An elevated blood concentration of nonesterified fatty acids is one of the indicators of NEB in cattle among others like increased β-hydroxy butyrate concentration, and decreased concentrations of glucose, insulin, and insulin-like growth factor-I.

2.5 Ketosis and metabolic profiles

Zhang et al. (2012) reported that nine of the 25 metabolites; namely, lactic acid (LA), glucuronic acid (GLCA), L-alanine (L-ala), glycolic acid (GA), ribitol, pyroglutamic acid (pGlu), galactose (Gal), 2,3,4-trihydroxybutyric acid (THBA) and glucose (Glc), decreased from low to high in both clinical ketotic and sub-clinical ketotic compared to control cows. Furthermore, 16 of 25 metabolites; increased in both clinical ketotic and sub-clinical ketotic compared to control. These metabolites were mainly 3-hydroxybutyric acid (BHBA) and nonesterified fatty acids (NEFAs), including palmitic acid (PA), heptadecanoic acid (HA), stearic acid (SA), trans-9-octadecenoic acid (T-9-OA), myristic acid (MA) and cis-9-hexadecenoic acid (C-9-HA), which belong to the families of ketone bodies, long chain unsaturated fatty acids, and saturated acids.

Li et al. (2014) compared plasma metabolic profiles of clinical ketotic Holstein dairy cows with control cows using liquid chromatography-mass
spectrometry (LC/MS). They concluded that compared to control cows, the levels of valine, glycine, glycocholic, tetradecenoic acid, and palmitoleic acid increased significantly in clinical ketosis. On the other hand, the levels of arginine, aminobutyric acid, leucine/isoleucine, tryptophan, creatinine, lysine, norcotinine, and undecanoic acid decreased markedly.

Sun et al. (2014) reported (1) H-Nuclear magnetic resonance based profiling of 25 metabolites in dairy cows with clinical and sub-clinical ketosis. Among the 25 metabolites, 4 were upregulated, 7 were downregulated and 14 were both upregulated and downregulated. The expression levels of plasma His, Glutamate, Gln, Lys, Phe, blood glucose, and lactate were significantly downregulated in cows with ketosis. The expression levels of myo-inositol, formate, citrate, Ala, Pro, Tyr, low-density lipoprotein (LDL) and very low density lipoprotein (VLDL) were downregulated only in cows with clinical ketosis. However, the expression levels of acetate, BHBA, acetoacetate and acetone were significantly upregulated in cows with ketosis. Choline, creatine, Gly, Leu, Ile and Val were upregulated only in cows with subclinical ketosis.

2.6 Oxidative stress

Pintea et al. (2008) carried out to assess the activity of antioxidant enzymes, the level of lipid peroxidation at the level of serum antioxidants. The high level of malonaldehyde (MDA) in the first week after parturition correlated with a low activity of antioxidant enzymes (GPX and catalase) and a low level of antioxidants.

Sahoo et al. (2009) observed in erythrocytic catalase activity a significant (P < 0.05) reduction in the group II after treatment but there was no significant increase in catalase activity in non-treated positive control (PC) animals in investigation for assessment of the erythrocytic oxidative stress indices after treatment of subclinically ketotic lactating cows.

Sharma et al. (2011) studied on oxidative stress and antioxidant status during transition period in dairy cows considering plasma level of catalase and glutathione (GSH) as antioxidants. The lipid peroxidation was significantly (p<0.001) higher in cows during early lactation as compared to the cows in advanced pregnancy. A significant positive correlation (r =+0.831, p<0.001) was found between MDA and catalase in early lactating cows. In early lactating cows, blood glutathione was significantly lower than in advanced pregnant cows.
Zhang et al. (2011a) found that in cows with subclinical ketosis, serum BHBA and NEFA concentrations were significantly higher, and glucose concentrations were significantly lower as compared to the values in healthy cows and no significant difference was observed in serum GSH-Px and catalase between the subclinical ketotic and healthy cows.

Shi et al. (2014) reported significant increase in levels of oxidative indicators (MDA and NO), whereas the levels of antioxidation indicators (GSH-Px, CAT and SOD) were markedly decreased in hepatocytes with increased BHBA levels.

Maurya et al. (2015) conducted the effect of vitamin E and zinc on oxidative stress and antioxidant enzymes during transition period in Karan Fries cows. The activity of plasma catalase and glutathione peroxidase were significantly lower in treatment as compared to control cows.

El-Deeb and El-Bahr (2017b) observed a significant increase in level of serum enzymes (AST and GGT) and oxidative stress biomarker (MDA and NO) in ketotic cattle as compared to control. A significant decrease in glucose, cholesterol, TAG and reduced glutathione were detected in ketotic cows as compared to control in an investigation of biochemical marker for ketosis in dairy cattle.

2.7 Fat to Protein Ratio

Heuer et al. (1999) reported that milk fat concentration tends to increase and milk protein concentration tends to decrease during postpartum negative energy balance. A fat to protein ratio of >1.5 in first day teat milk is indicative of a lack of energy supply in the feed and of risk for ketosis.

The effect of F/P ratio has been reported for dairy cattle in several studies (Toni et al., 2011; Buttcheureit et al., 2012; Zink et al., 2014). Butcheriet et al. (2012) reported that fat-to-protein ratio (F/P ratio) and body condition score are potential variables to describe how well cows can adapt to the challenge of early lactation. The increase of F/P ratio in the early lactation was also significantly associated with increase of risk of being culled from the herd (Toni et al., 2011). Moreover, the F/P ratio is a valuable indicator of negative energy balance in postpartum cows and might be helpful in selecting concerning metabolic or other disorders (Zink et al., 2014).

Jenkins et al. (2015) reported that the optimized cut-off of FPR (fat to protein ratio) is 1.42 with sensitivity (Se) of 92% and specificity (Sp) of 65%. The objective was to identify a fat-to-protein ratio (FPR) cut-off to diagnose subclinical ketosis.
Review of Literature (SCK) and to evaluate the effect of propylene glycol (PPG) treatment of cows with high FPR.

Kayano and Kataoka (2015) reported that milk yield (kg/day/cow) and protein-to-fat (P/F) ratio in milk were significant factors (P<0.05) for the diagnosis of ketosis in multiparous cows. However, For primiparous cows, lactose content (%), solid not fat (SNF) content (%) and milk urea nitrogen (MUN) content (mg/dl) were significantly associated with ketosis (P<0.01).

2.8 Somatic Cell Count

Al-Rawashdeh (1999) studied bovine ketonemia among 1155 dairy cows in various stages of lactation and parity on 25 Jordanian dairy herds, and showed that serum concentration of β-hydroxy butyrate (BHBA) <0.9 mmol/L between 0.9 and 1.7 mmol/L and >1.7 mmol/L were considered to indicate normal, mild and severe ketonemia, respectively. The point prevalence of mild and severe ketonemia were 22 and 3.8 per cent, respectively and the prevalence of ketonemia decreased with increasing herd size. Further, a non significant association was found between the prevalence of ketonemia and parity, stage of lactation, metritis, somatic-cell count (SCC) and serum cholesterol levels.

Carrier et al. (2004) reported that Somatic cell counts greater than 1 million cells/mL will cause an elevation in reading of both the BHBA strip tests.

2.9 Ketone Bodies in Urine and Milk

Nielen et al. (1994) stated that sensitivity and specificity of the Nitroprusside powder test with milk in various studies is 28-90% and 96-100%, respectively.

Tanwar et al. (2005) tested urine for the presence of ketone bodies by Rothera’s test and urine reagent strip and recommended urine test as one of the important parameters for diagnosis of subclinical ketosis.

Rukkwamsuk et al. (2008) analysed urine samples for ketone bodies using the sodium nitroprusside test within 24hrs after collection and stated that results of sodium nitroprusside test for ketone bodies in urine were highly correlated with the concentration of BHBA in serum of dairy cows.

Biswa et al. (2016) studied prevalence of ketosis in 2760 cows based on qualitative assessment of ketone bodies in urine (Rothera’s test) and milk (Ross test). The incidence rates for clinical were found to be 25.2%, 26.6% and 30.3% in
crossbred Jersey cows, crossbred Holstein Friesian and indigenous cows, respectively. The incidence rates for subclinical ketosis were found to be 12.2%, 11.2% and 2.9% in crossbred Jersey cows, crossbred Holstein Friesian and indigenous cows, respectively. The breed wise overall prevalence rate was recorded to be 38.0% in crossbred Jersey cows, 37.8% in crossbred Holstein Friesian and 33.2% in indigenous cows.

A total of 350 post parturient cows having history of anorexia and drop in milk yield were screened for clinical ketosis (CK) and sub-clinical ketosis (SCK) using Rothera’s test and urine Diastix and forty (11.42%) cows were diagnosed as ketotic (Kumar et al., 2016).