2 Literature Review

2.1 Fatty liver

Over 25 year ago, **Morrow (1976)** described fatty liver as a clinical condition which developed in obese dairy cattle and caused severe health problems at parturition. Then, **Reid and Roberts (1983)** and **Stöber and Scholz (1991)** stated that fatty liver in dairy cattle was mainly caused by a discrepancy between energy requirements for milk production and feed intake at early lactation. It occurred with high incidence in high producing dairy herds with its considerable consequences on health and productivity and was considered one of the most important metabolic disorders in cattle.

Oetzel (2001) defined fatty liver syndrome as a type II ketosis which is diagnosed in a dairy herd by the presence of a high incidence of sub-clinical or clinical ketosis in cows at the first two weeks of lactation, associated with a high prevalence of elevated blood NEFA (non-esterified fatty acid) concentrations in the pre-fresh cows. Moreover, there were some factors which helped the diagnosis of these cases, including obesity, very persistent ketosis, high rates of displaced abomasums, and high mortality rates in early lactation.

From an economic point of view, **Byers (1999)** mentioned that fatty liver was generally complicated by other diseases such as displaced abomasums, retained placenta and ketosis. The outstanding clinical features for these diseases are the poor response to treatment of the accompanying disease and cows might die or recover slowly.

2.1.1 Incidence of fatty liver syndrome

Morrow (1976) reported that the fat cow syndrome occurred sporadically and depending on feed management practices. Moreover, it was most frequent in loose housing where cattle in all stages of lactation including dry cows occasionally were fed and managed in one group.

Fatty infiltration of the liver is part of a generalized fat mobilization syndrome which occurs either in late pregnancy when the nutrient intake is decreased in dairy cows that are previously

well fed and in good body condition or in early lactation particularly in highly yielded dairy cattle **(Radostitis et al., 2000)**.

Reid and Roberts (1983) stated that the incidence of fatty liver was higher in highly lactating cattle than in heifers because milk production outstrips appetite and body reserve were used to meet the energy deficit which occurred in highly producing animals. The authors added that values of hepatic fat in heifers were below 10% at one week after calving while the fat content of highly lactating cattle was more than 20%. Furthermore, **Reichel et al. (1989)** concluded that the incidence of fatty liver increased with the number of calvings.

Gröhn et al. (1983) recorded that in about 30% highly producing cows, the infiltration of fat in livers was severe and associated with reversible but significant effects on liver structure and function. On the other hand, in some populations of cows, the incidence of fatty liver was much lower and insignificant.

Gerloff and Herdt (1984) stated that the disease could occur in non-lactating dairy cows by imposition of a partial starvation diet in late pregnancy in an attempt to reduce the body weight of cows and avoid dystocia.

In a clinical investigation of a dairy herd with fat cow syndrome, **Morrow et al. (1979)** found that the herd problem was characterized by 82% morbidity rate and 25% mortality rate during a 4 months period.

Fatty liver is associated with several post-parturient diseases including ketosis, retained placenta, mastitis and metritis. Impaired hepatic function attributable to fatty infiltration was suggested to result in metabolic disturbance, reduced fertility and suppressed immune competence, thereby rendering cows became more susceptible to reproductive and infectious diseases **(Gerloff et al., 1986)**. Moreover, **Rukkwamsuk et al. (1999)** and **Heuer et al. (2000)** showed that fatty liver in cattle might be associated with an increased incidence of metabolic disorders.

Grummer (1993) and **VanWinden et al. (2003)** reported that ketosis and left abomasum displacement were two major metabolic disorders that developed during the post-parturient period, therefore they considered them to be closely related to fatty liver. In addition to that,

Komatsu et al. (2002) found fatty liver in 55% of liver samples obtained from cows with abomasum displacement by using histopathological and clinical chemistry methods. In dairy cows with abnormally long dry periods had a tendency to become obese and develop the fatty liver syndrome at parturition **(Reid and Roberts, 1983; Radostitis et al., 2000)**. Furthermore, **Oetzel (2001)** concluded that obese cows were also prone to increased adipose sensitivity to mobilize body fat very rapidly under conditions of stress or negative energy balance.

Treacher et al. (1986) and **Van den Top et al. (1996)** documented that cows with excess body weight or over-condition at calving were likely to have lower feed intake post-partum than cows that had normal body weight or normal condition. Therefore, **Herdt et al. (1988) and Armentano et al. (1991)** found that cows in good body condition ante-partum had greater risk of metabolic problems because of excessive mobilization of body reserves. On the other hand, **Busato et al. (2002)** concluded that the best metabolic status existed in cows with a body condition score >3.25 ante-partum, especially when post-partum nutrient and energy intake were sufficient.

Wentink et al. (1992) demonstrated that hepatic lipidosis might occur in exceptional circumstances when the energy supply to animals in their last months of pregnancy was insufficient. Furthermore, the authors added that animals carrying twins were extremely vulnerable to a shortage of energy in the last months of pregnancy.

Byers (1999) stated that the transition period (3 weeks before to 3 weeks after calving) is a critical time in the life of dairy cows. During this time, the modern dairy animal makes a great metamorphosis. She moves from a dry cow with marginal nutrient requirements to a lactating cow with massive metabolic needs therefore this transition affects her health, production and reproduction traits.

2.1.2 Etiology

Fat is mobilized from body reserves as shown by the rise in liver fat, which occurs two to three weeks before calving. The mobilization is probably due to a change in the hormonal environment when the cow approaches calving. After calving, the metabolizable energy (ME) intake lags

behind the ME requirements. The cow is still in negative energy imbalance until 10 to 12 weeks after calving. In order to balance the energy, the cow mobilizes body reserves which leads to loss of body weight and condition and develops fatty liver syndrome **(Reid and Roberts, 1983)**.

Previous studies showed that both a rapid increase in milk production by either selection or breeding and sudden changes of food from a roughage based ration in the dry period to an energy rations, immediately after calving in association with poor rumen adaptation **(Goff and Horst, 1997)** and an occasional excess intake of digestible crude protein **(Hibbitt et al., 1969)** might lead to lower dry matter intake or sub-optimal rumen fermentation, with the result of deficient energy.

In Western Australia, **Allen (1981)** found that fatty liver syndrome resulted from ingestion of lupin stubbles especially in late pregnant cows which might be due to the development of secondary nutritional ketosis resulting from mild inappetance caused by the lupinosis toxin.

Gerloff et al. (1986) indicated that stress induced by environmental conditions near calving might promote TG accumulation during this period.

In many dairy farms in Slovakia, **Reichel et al. (1989)** found that disturbance in dry matter, crude fiber intake and ruminal indigestion during the lactation curve and reproductive phase would increase incidence of fatty liver. Then, **Bertics et al. (1992)** mentioned that the gradually increases plasma non-esterified fatty acid during the final days of pregnancy and peakes at parturition and early lactation might be explained by the gradual depression of dry matter intake observed during these times. Moreover, **Herdt (1988)** explained that increases in plasma non-esterified fatty acids at calving might be due to a change in the endocrine status.

Schulz (1985) concluded that post-parturient hypophosphataemia played an important role in the pathogenesis of fatty liver and he advised to supply phosphorus as prophylaxis and therapy for post-partum fatty liver.

Pullen et al. (1990) stated that fatty liver occurred when the rate of hepatic triglyceride (TG) synthesis exceeded the rate of TG disappearance through either hydrolysis or secretion via very low-density lipoprotein (VLDL). On the other hand, ruminants had a very slow rate of hepatic

VLDL secretion relative to most species, which made them more susceptible to fatty liver syndrome. In addition to that, serum concentration of apolipoprotein B100, the major apolipoprotein of VLDL, was found to be lower in cows experiencing metabolic disorders **(Itoh et al., 1997)**.

Katoh et al. (1993) speculated that the reduced liver nuclear estrogenic receptor (ER) concentration attributable to interruption of estradiol (E_2) administration triggers leading to a reduction of liver apolipoprotein B-100, thus release of triglyceride from the liver stopped resulting in triglyceride accumulation in the liver and development of fatty liver.

Uchida et al. (1992) induced experimentally fatty liver in cows through administration of ethionine, which led to a decrease in serum apolipoprotein B-100 and A-I concentration, which is responsible for secreting accumulated triglyceride.

The enhanced mobilization of fatty acids from adipose tissue stores, caused by the drop in food intake after parturition, might be crucial in fatty liver development. In addition, the hepatic triacylglycerol accumulates in fatty liver because of the increased hepatic uptake of non-esterified fatty acids and the simultaneous increases in activity of diacylglycerol acyltransferase **(Van Den Top et al., 1995)**.

In dairy cows, **Rukkwamsuk et al. (1999)** stated that overfeeding during the dry period led to over-condition at calving and to depression of appetite after calving. Consequently, at calving over-conditioned high producing dairy cows inevitably went into a more severe negative energy balance post-partum than cows that did have a normal appetite. During the period of negative energy balance, the energy requirements of the cow were satisfied by lipolysis and proteolysis. Lipolysis resulted in an increase in the concentration of non-esterified fatty acid in the blood. In the liver, these fatty acids were predominantly esterified to triglyceride that was secreted in very low-density lipoprotein. In early lactation, the capacity of the liver of cows with a severe negative energy balance to maintain the export of triglycerides in the form of low density lipoproteins in comparison with the hepatic triglyceride production was not always adequate. As a result, the excess amount of triglyceride accumulated in the liver, led to fatty infiltration of the liver and developed fatty liver syndrome.

2.1.3 Pathogenesis

The increased lipolysis around partus is hormonally regulated and not primary an expression for energy deficit **(Morrow, 1976)**. **Bell (1980)** stated that the liver is an important site for removal of free fatty acids (FFA) from circulating blood plasma. After esterification to triglycerides, they are transported from the liver in very low-density lipoproteins (VLDL).

Katoh (2002) reviewed that non-esterified fatty acids (NEFA) were β-oxidized to acetyl-coenzyme A (acetyl-CoA) which was further oxidized in the tricarboxylic acid cycle (TCA) by binding with oxaloacetic acid. When oxaloacetic acid is exhausted for gluconeogenesis in early lactation and depleted in mitochondria, therefore acetyl-CoA couldn't enter the TCA and, instead, was directed toward the pathway for ketogenesis and development of ketosis. To adapt the acceleration of gluconeogenesis during fatty liver, amino acids such as aspartic acid are mobilized in excess from skeletal muscle (the major pool of amino acids). The abundant amino acids lost from skeletal muscle might induce downer cow syndrome.

Excessive deposition of fat droplets in liver cell interferes with normal liver function including metabolic disturbance, insufficient detoxifying activity and deficient cellular and antibody dependent defence **(Muylle et al., 1990)**. Moreover, **Strang et al. (1998)** found that triglyceride accumulation reduced ureagenic activity up to 40% and this might cause ammonia spillage into the pericentral portion of the liver and into the periphery, contributing to the etiology of several metabolic disorders.

Severe fatty liver is commonly paralleled by low leukocyte counts in the peripheral blood **(Reid et al., 1983a)**. Moreover, ketone bodies in sub-ketonic and ketonic concentrations are able to inhibit the phagocytic activity of bovine polymorphic nuclear cell isolated from milk and blood **(Klucinski et al., 1988)**. **Sato et al. (1995)** reported that reduction of the mitogen-induced lymphocyte blastogenesis in cows is associated with glucose deficiency or during ketosis.

Zerbe et al. (2000) reported that increased liver triglyceride content in fatty liver cows was associated with decreased functional capacities of polymorphnuclear neutrophilic granulocytes derived from blood and uterus. This might lead to an increase in the incidence of infectious disease such as endometritis and mastitis. In addition, prolonged toxaemia resulting from

bacterial infections could be present in cows with fatty liver as a result of insufficient clearance function of the liver **(Ohtsuka et al., 2001)**.

In vitro, **Homa and Brown (1992)** found that the non-esterified fatty acids inhibited the break down of germinal vesicles, which are considered the initial step in the resumption of meiosis in the oocyte. Moreover, **Rabiee et al. (1997)** suggested that fatty liver cows had some fertility problems because plasma non-esterified fatty acids could enter the ovaries and produce an inactive ovary.

Oetzel (2001) stated that fatty liver infiltration impaired both the gluconeogenic potential which greatly increases a cow's risk for ketosis once lactation starts as well as the immune function. The net result, that a cow is not only persistently ketotic, but also immune suppressed and many cows might die from infection (metritis, mastitis and pneumonia).

2.1.4 Clinical signs

West (1990) suggested that the percentage of fatty infiltration of the liver is significantly correlated with the degree of clinical illness and classified clinical disease into four grades. On the other hand, **Catania and Renninger (2003)** showed that there was little correlation between fatty degeneration and clinical signs until the disease was marked and liver samples would float in distilled water.

Morrow et al. (1979) stated that clinical sings of fatty liver included anorexia, depression, weakness, ketonuria, fever, marked decrease in milk production, and progressive debilitation. Moreover, development of ketosis and retained fetal membranes were found in 38% and 62% of the affected cows, respectively.

Herdt et al. (1983) classified the degree of fatty liver according to the fat content estimation by the copper sulfate test into severe, moderate and mild fatty livers and they found severe fatty liver accompanied with general systemic disturbance. Moreover, **Gerloff et al. (1986)** categorized cattle into having mild, moderate and severe hepatic lipidosis on the basis of maximal amounts of hepatic triglyceride that accumulated in the liver during the peri-partum period. Rate of disease, culling and death rate related to disease were greater in cattle with severe hepatic lipidosis.

The general signs of fatty liver includes extreme obesity with a body weight of 680 to 820 kg for Holstein cows, decreased resistance to infection and an increased number of peri-parturient diseases **(Morrow, 1976)**. Moreover, **Radostitis et al. (2000)** cited that the signs of fatty liver included depression, anorexia, ketonuria, marked decrease in milk production, progressive debilitation, weakness and elevated temperature due to associated infectious diseases. In addition to that some cattle develop nervous signs such as staring gaze, holding the head high and muscular tremors of head and neck.

Reid and Roberts (1983) found that sub-clinical fatty liver was associated with extension of the calving to conception interval due to delays in the onset of cyclic ovarian activity and normal oestrus cycle after calving and due to reduce conception rate. **Jorritsma et al. (2000)** concluded that fatty liver in dairy cows decreased the probability of pregnancy (30%) and also the probability of esterus was low (35%). Moreover, the authors added that the intervals between parturition and first heat and parturition and the time of next pregnancy would increase. The data indicated also that an increase in milk production had no negative impact on fertility as long as the amount of triacylglycerol in the liver remained the same.

In naturally affected pregnant cows with hepatic lipidosis, **Wentink et al. (1992)** mentioned that the clinical signs of disease were lethargy, anorexia, diarrhoea and sternal recumbancy.

Fatty liver is associated with a decrease in the thickness of back fat and there is a good correlation (r=0,72) between measurements of back fat thickness and liver fat content, which indicates an increase in the lipolysis rate **(Staufenbiel et al., 1993)**.

2.1.5 Clinical pathology

In fatty liver syndrome, **Morrow (1976)** found that the urea nitrogen of blood might be elevated about 20mg/100ml and glucocorticoid typically being below the normal value of 13 to 20ng/ml, blood glucose below 40mg/100ml and free fatty acids above 7mg/100ml while triglyceride was depressed below 5mg/100ml. Ornithine carbamyl transferase (OCT) and sorbitol dehydrogenase (SDH), which are considered specific enzymes for liver function, were above 200 and 500

units/ml respectively. In addition to that **Morrow et al. (1979)** added that affected cows had leucopoenia.

Reid et al. (1983a) concluded that cows with moderate to severe fatty liver (>20% fat in liver) had a significantly reduced total leukocyte count compared with cows with mild fatty liver (<20%fat) mainly due to a reduction in the numbers of circulating neutrophils, lymphocytes and eosinphils. Moreover, the percentage of T-lymphocytes was unaltered but the percentage of immature neutrophils was increased in cows with moderate to severe fatty liver. Experimentally, the author added that the ability of neutrophils to kill *Staphylococcus aureus* was not impaired in cows with moderate to severe fatty liver whereas mobilization of leucocytes into milk in response to *E. Coli* cultural filtrate challenge was significantly impaired. Then, **Reid et al. (1984)** mentioned that there was a negative correlation between the fat content of the liver and the total white cell count. On the other hand, there was no correlation between liver fat content and percentage of lymphocytes, packed cell volume and haemoglobin.

Gerloff and Herdt (1984) found that the fatty liver, which resulted from dietary restriction in non-lactating cows, was associated with a depression of white blood cell counts and mildly increased serum aspartate transaminase (AST) activity. Moreover the authors stated that aspartate transaminase (AST) activity was the only laboratory finding that was consistently correlated with hepatic fat infiltration due to a considerable lipid infiltration in the muscle as well as the liver, so that high AST activity might reflect muscle as wall as liver damage. Alternatively, AST, Sorbitol dehydrogenase (SDH) or other liver enzymes did increase.

Fatty cow syndrome is characterized by significantly decreases in glucose, insulin, albumin and magnesium. On the other hand, there is a significant elevation of non-esterified fatty acids and bilirubin **(Reid, 1986).**

West (1990) discovered that bromosulphthalein (BSP) excretion in cows with fatty cow syndrome was considerably slower than in healthy non-pregnant cows and plasma BSP concentration was greater than 0,1mg/100ml at 60min.

Schäfer et al. (1991) concluded that fat concentration beyond 10% was accompanied by higher ketone body and increased bilirubin levels as well as AST activities.

Staufenbiel et al. (1991) mentioned that glutamate dehydrongenase (GLDH) activity increased in case of fatty liver syndrome due to destruction of liver cells.

Pechova et al. (1997) showed that cows suffering from hepatic steatosis had a high concentration of free fatty acids as compared to controls (0.598±0.319 vs. 0229±0.017mmol/l), total bilirubin (6.230±2.97 vs. 4.030±1.24µmol/l), AST (1.82±0.528 vs. 1.21±0.195µkat/l), LD (28.76±7.14 vs. 20.81±1.84µkat/l), oxidized ketone bodies (0.346±0.280 vs. 0.176±0-015mmol/l) and χ globulins (34.57±12.36 vs. 29.52±12.27g/l). On the other hand, the concentration of triglyceride (0.027±0.014 vs. 0.113±0.09mmol/l) and the A/G ratio (0.597±0.192 vs. 0.691±0.166) were lower.

Rehage et al. (1999) concluded that the measurement of the serum bile acid concentration revealed little information about the degree of hepatic lipidosis in dairy cattle.

In experimentally induced fatty liver in cows **Zhu (2000)** found that hepatic triglyceride accumulation might inhibit ureagenesis and resulted in increasing circulating ammonia, glutamine and urinary ammonia nitrogen but didn't affect blood pH homeostasis. Moreover, vitamin E and selenium have been found to be low in cattle with fatty liver **(Catania and Renninger, 2003)**.

2.1.6 Diagnosis

Morrow (1976) stated that the diagnosis of fatty cow syndrome was based on a history of excessive energy intake as in the case of obese cows and also on the presence of one or more peri-parturient conditions such as milk fever, ketosis, displaced abomasum, retained fetal membranes and/or mastitis. The author added that the diagnosis frequently might be confirmed by a unfavourable response to conventional treatment. For effective treatment of the same condition in cows with normal physical condition, the prognosis in this case was guarded to be poor.

Katoh (2002) concluded that apoB-100; apoA-I and apoC-III lipoprotein serum concentrations and Lecithin-cholesterol acyltransferase (LCAT) activity were useful markers for early diagnosis of fatty liver. In addition to that, monitoring of these apoprotein concentration and enzyme activity during the peripartum period were highly valuable to detect susceptibility to metabolic, reproductive and inflammatory diseases.

Heuer et al. (2000) suggested that daily milk yield and the fat/protein ratio were more reliable indicators for prediction of metabolic diseases and fertility problems than the post-partum body condition score or a decrease in the body condition score.

2.1.6.1 Liver biopsy and analysis

Muylle et al. (1990) concluded that blood parameters did not give sufficient evidence for conclusions about the metabolic profile of the liver.

Simpson (1985) stated that liver biopsy was recognized as a valuable diagnostic tool in many species of animals when altered liver function tests suggested hepatic disease to be present. Moreover, the histological examination of liver biopsies allows the type and the extent of any pathological changes to be precisely assessed, thus improving the accuracy of the diagnosis and allowing more specific treatment to be undertaken.

Monaghan and Sheahan (1987) suggested that liver biopsy was a fast, safe and reliable method for detection sub-clinical disease with especial reference to an outbreak of *Senecio* species poisoning in cattle. Moreover, in sheep, **Harvey et al. (1984)** concluded that the serial liver biopsy procedure causes negligible alteration in the haematological, histological or serum biochemical parameters.

Swanson et al. (2000) reported that liver samples obtained from live animals could be used to help in diagnosing diseases or metabolic disorders and to determine the liver concentration of nutrients, drugs or other compounds without killing the animal. Furthermore, **Radostitis et al. (2000)** cited that a liver biopsy could be used to determine the severity of fatty liver through

estimation of total lipid and triglyceride. Therefore, liver biopsy is considered the most reliable method of accurately estimating the degree of fatty infiltration. Moreover, **Staufenbiel et al. (1993)** found that fat content clearly could be defined exclusively from investigations of liver samples rather than from the analysis of blood or milk parameters.

Herdt et al. (1983) classified fatty liver into clinically healthy (less than 13%), mildly affected (greater than 13%), moderately affected (greater than 25%) and severally affected (greater than 34%) classes according to the buoyancy of liver biopsies in copper sulfate solutions with different specific gravity (1,055 and 1,055) and water (1,000). Moreover, **Collins et al. (1985)** mentioned that fatty liver in dairy cattle can be classified according to histological measurements of the fat content in liver biopsies by using toluidine blue and oil red into mild (<20vs >24), moderate (20 to 40 vs. 24 to 48) and severe (>40 vs. >48).

West (1990) suggested that there exists a significant relationship between the severity of fatty liver and the fat content of livers. On the other hand, **Gaal et al. (1983)** revealed that the estimation of the hepatic total lipid content is not considered an acceptable alternative method for assessing fatty infiltration because the high basal level of non-triglyceride lipid masks the increase in the hepatic triglyceride content, which is characteristic of fatty liver. In addition to that, **Staufenbiel et al. (1993)** found that there exists a great variability in the liver fat content, from 3.9% to 24%, in the peripartum period which leads to no strong reference values to distinguish between physiological and pathological livers.

Gerloff et al. (1986) stated that cattle with severe hepatic lipidosis had a greater concentration of hepatic triglyceride before calving and after parturition. Cattle were divided retrospectively into mild (<50mg/g liver), moderate (50 – 100mg/g liver) and severe (>100mg/g liver) hepatic lipidosis accordingly on the basis of maximum hepatic triglyceride.

Pechova et al. (1997) reported that the concentrations of triglyceride of control cows and of cows suffering from hepatic steatosis were 0,027±0,014 and 0,113±0,09 mmol/l, respectively. Moreover, **Jorritsma et al. (2000)** recorded that cows with a high liver triglyceride content (>50%) had more frequant fertility problems than cows with a low liver triglyceride content (<50%). **Katoh (2002)** reviewed that the hepatic triglyceride contents in healthy cows during the

peripartum period were less than 30mg/g of liver (wet weight) and fatty liver subsequently was defined as a liver having more than 30mg/g.

2.1.6.2 Histopathological examination

Craig et al. (1991) stated that histopathological examination of liver biopsies and liver enzymes are the best means of ante-mortem diagnosis of chronic and chronic delayed liver diseases.

Gröhn and Lindberg (1982) stated that a liver biopsy taken only from the living animal could give reliable microscopical information about the condition of the liver. Then **Gaal et al. (1983)** used a stereological estimation of the fractional volume of fat in hepatocyte as a reliable methods for assessing fatty infiltration of the liver in dairy cows.

In histological measurements of the fat content by either simple ORO (Oil Red O) or TOLB (Toluidine Blue) methods, **Collins et al. (1985)** concluded that exists a strong correlation between these methods. Therefore, the simple ORO method is an acceptable alternative in the routine histological examination to the tedious and technically demanding TOLB method. In addition, fat estimation using frozen sections stained with ORO **(Reid, 1986)** or Sudan III **(Romsis, 1989)** were histological methods of choice for most routine purposes.

Komatsu et al. (2002) concluded that the percentage of PAS (periodic acid Schiff) negative samples were significantly larger in case of severe fatty degeneration.

Carlton and McGavin (1995) cited that in mild fatty liver, the lipids might accumulate only in specific portions of each lobule such as the centrilobular but lobules in extreme cases, the entire liver is affected. Moreover, **Johannsen et al. (1991)** found that the histological picture of severe fatty liver is characterized by diffuse fatty infiltration and moderate to great lipid vacuoles while a moderate degree can be seen only peripherilobular and vacuoles varying from small to moderate size.

Morrow et al. (1979) and **Reid (1986)** described that histological changes of diffuse fatty liver, are characterized by the presence of large fat vacuoles that might develop to fat cysts especially

around the central vein and enlarged liver cells. Damage of mitochondria led to decreased protein synthesis. In addition, the nuclei of most hepatocytes were compressed against the cell membrane.

Smith (1996) and **Radostitis et al. (2000)** mentioned that the macroscopic picture of a fatty liver was a grossly enlarged, pale, yellow and friable liver and liver tissue could float above the surface of water.

Sevinc et al. (2003) classified fatty livers histologically according to the mean percentage of fat in the liver into severe (43.2 ± 3.5%), moderate (15.2 ± 0.6%) and mild (6.2 ± 2%) classes.

2.1.6.3 Ultrasonographic examination of the liver

Baker and Dalrymle (1978) stated that diagnostic ultrasound imaging was a non-invasive, safe method for assessment of structures and tissue consistency in various organs. There exists no biohazard in diagnostic ultrasound, being reported for other diagnostic methods.

Ultrasonography had been routinely used for about 18 years as diagnostic procedure in dogs **(Nyland and Hager, 1985)** and horses **(Rantanen, 1986)** for diagnosis of liver diseases. The first researchers who studied the ultrasonography of livers in cows were **Itabisashi et al. (1987)** who described the ultrasonographic picture of hepatic abscesses in cows experimentally inoculated by *Fusobacterium necrophorum*.

Yamaga and Too (1984) mentioned that the best site for ultrasonographic imaging of the liver in cattle was on the right side between the 8^{th} to 12^{th} intercostal space ventral to the level of the shoulder joint. The authors added that the normal hepatic imaging was a wedge shape pattern and had comparatively low amplitude and multiple fine echo patterns. In addition, the portal vein appeared as a star shaped configuration with surrounding echogenic definition, while the hepatic vein appeared as an oval structure without echogenic wall. Moreover, **Braun (1990)** stated that a 3,5MHz transducer was most suitable for ultrasonographic examination of bovine livers because of giving greater depth of penetration and better visualisation of most internal structures.

In a hepatic ultrasonographic examination of 180 cows of different ages and breeds, **Braun and Gerber (1994)** revealed that there was a significant correlation between body weight; milk production and stage of pregnancy with the ultrasonographic measurement of liver size, gall bladder, caudal vena cava and portal vein, while, breed and age had no effect.

Some research was done on usage of the ultrasound for diagnosing liver disease in cattle. **Lechtenberg and Nagaraja (1991)** made ultrsonographic observations on bovine hepatic abscesses induced experimentally by the inoculation of *Fusobacterium necrophorum* into the portal vein, while, **Liberg and Jösson (1993)** and **Braun et al. (1995)** used ultrasound image for diagnosing naturally developed liver abscesses.

Braun et al. (1994) and **Braun et al. (1995)** indicated that an ultrasonographic examination of livers was a valuable aid in the diagnosis of cholestasis and cholangitis. In addition to that, **Braun et al. (2000)** and **Mohamed et al. (2002)** pointed out that percutaneous ultrasound guided needle aspiration and catheterization of the portal and hepatic veins were safe procedures in cattle as long as they were performed carefully.

Acorda et al. (1994a) suggested that different echo patterns like bright pattern, deep attenuation and blurring of hepatic vessels and edges could be used to distinguish various diffuse hepatocellular disorders in dairy cattle. Therefore, ultrasonography can be used as a screening test before using more invasive procedures.

Acorda et al. (1994b) concluded that digital analysis of hepatic ultrasonograms could be useful in the evaluation of the degree of fatty infiltration of livers in dairy cattle. In addition, **Acorda et al. (1995)** recommended the usage of a combination of ultrasonogram and digital analysis of hepatic ultrasonograms instead of biochemical analysis in the diagnosis of fatty infiltration of livers in dairy cattle.

Braun et al. (1996) showed the ultrasonographic features of fatty livers which were an increase in the size of liver, round margins, hyperechoic hepatic parenchyma near the abdominal wall, a weak echo as distance increases from the abdominal wall and poor visualization of hepatic blood vessels.

2.2 Hypophosphatemia

Forrester et al. (1989) mentioned that low concentration of serum inorganic phosphate (<2.5 mg/dl), has not been reported commonly in veterinary medicine. However, in humans hypophosphatemia is associated with several conditions (e.g., diabetes mellitus, alcoholism, respiratory alkalosis) and is responsible for significant morbidity and mortality. Recent reports of hypophoshatemia-induced disease in the veterinary literature had prompted interest into the effects of hypophosphatemia on veterinary patients. Consequences of severe hypophosphatemia in veterinary patients might include haemolytic anaemia, seizures, altered mentation, cardiomyopathy, and skeletal muscle weakness.

2.2.1 Function of phosphorus

In 1918, **Osbrone and Mendal (1918)** found that phosphorus was necessary for normal growth of rats. After that, **Teiler et al. (1924)** reported on the importance of phosphorus supplementation in the nutrition of growing and breeding cattle on ranges in South Africa.

Swenson (1984) reported that phosphorus had more known function in the body than other mineral elements. In addition to uniting with calcium and carbonate to form compounds that increase the rigidity of bones and teeth. Furthermore, phosphorus is found in every cell of the body and is vital part of much of the metabolic process.

Phosphorus (P) is an essential element. Approximately 80% of total body phosphorus is found in the inorganic portion of bones **(Underwood, 1981)**. Moreover, **Radostitis et al. (2000)** cited that phosphorus is essential for the laying down of adequately mineralized bones and teeth and its deficiency would lead to their abnormal development.

Goff (2000) reviewed that phosphorus is a component of phospholipids, phosphoproteins, nucleic acids, and energy-transferring molecules such ATP. Phosphorus is an essential component of the acid-base buffer system.

Phosphorus exists in the body as inorganic phosphate and as organic phosphate esters (**Young, 1980)**. **Knochal (1977)** added that most intracellular phosphorus is in the organic form, while most inorganic phosphate is located extracellularly.

Forrester et al. (1989) reviewed that organic phosphorus was present in many compounds in the body including: - 1-Phospholipid (lecithin) as a component of the external plasma membranes and intracellular membranes that form organelles, which play an essential role in maintaining cellular integrity. 2- Phosphoproteins are component of mitochondria used in the electron transport system. 3- Nucleic acids such as cyclic 3,5- adenosine monophosphate (cAMP) serve as second messenger in mediating the action of more than ten hormones. 4- Adenosine triphosphate (ATP) contains three phosphate radicals and is energy source for several physiological processes including the sodium-potassium pump, the synthesis of chemical compounds and muscle contraction.

Fraser et al. (1987) stated that inorganic phosphate is a substrate for many vital functions of the body including oxidative phosphorylation, production of 2,3-DPG in erythrocytes, and glycogenolysis in the liver and kidney. Moreover, it also regulates the rate of glycolysis and acts as a co-factor in many enzyme systems.

The physiological roles of phosphorus concern many metabolic processes, play a major role with calcium in the formation of bones and teeth, maintain osmotic and acid-base balance, are components of nucleic acids which are important in genetic transmission, exhibit control of as cellular metabolism activator or are components of some enzyme systems, for energy transfer inside living cells through either low energy phosphates (glucose-6-phosphate or tri- phosphate) or high energy phosphate bonds (ATP, GTP, CTP, UTP, ITP) and play an essential role in metabolisms of carbohydrates and fatty acids **(Martin and Davenport, 2002)**.

Goff (2000) mentioned that salivary phosphate secretion acts as buffer system inside the rumen and supplies rumen microbes with a readily available source of P, which appears necessary for cellulose digestion.

Radostitis et al. (2000) recorded that inorganic phosphate plays an important role in the metabolism of carbohydrates and creatinine in chemical reactions occurring in muscle contraction.

2.2.2 Homeostatic control of phosphorus

Care et al. (1980) mentioned that a strong correlation exists between the serum phosphorus P level and salivary phosphorus through reducing salivary P during hypophosphatemia and increasing in excess phosphorus intake.

Plasma inorganic phosphorus concentration is normally between 4-8 mg/dl. About 1-2g P is present in the plasma inorganic P pool, and 4-7g of P is normally present in the intracellular P pool of a 500 kg cow. Intracellular P content is about 78mg/dl and total body intracellular P content is about 155g with 5-6 of those grams located within erythrocytes. Maintaining the intracellular P pool involves replacing P removed for bone and muscle growth, for endogenous faecal loss, urinary P loss and milk production, with P absorbed from the diet or resorbed from bones **(Reinhardt et al., 1988)**.

Forrester et al. (1989) reported that phosphorus was distributed throughout the body with 80% to 85% present in the skeleton as hydroxyl peptide, a major component of bones. Approximately 9% to 15% are located in skeletal muscle and the rest in the soft tissues. It is considered the major intracellular anion. Three organs maintain the phosphorus homeostasis: the small intestine, kidney and skeleton. Intestinal absorption of phosphate is an active, energy dependent process and an increased absorption occurs with a decreased dietary calcium and increased acidity of intestinal content. Moreover, growth hormones enhance intestinal absorption and renal reabsorption of phosphate and vitamin D stimulates intestinal phosphate transport.

During gestation, **House and Bell (1993)** reported that fetal skeletal development could withdraw up to 10g of P per day from maternal P pools. However, **ARC (1980)** recorded that about 0.3g of P was incorporated into each kg of body tissue (muscles) gained during growth of the animal and 1g was essential for production of 1 kg milk.

Serum inorganic phosphorus is affected by several factors as mentioned by **Forar et al. (1982)** like age of animal, milk yield, stage of pregnancy, season of year, breed, feeding pattern and dietary phosphorus. **Ogawa et al. (1987)** added that hypophosphatemia develops not only because of dietary deficiency of phosphorus but was also probably caused by an overall disorder of phosphate modulation associated with various factors, involving high milk production, inappetance, stress and hormonal changes after calving.

The factors affecting phosphorus absorption and its availability are vitamin D deficiency and diets containing iron, aluminium and unsaturated fatty acid **(Janson et al., 1983)**. Furthermore, **Mudgal and Ray (1967)** and **Leibholtz (1974)** mentioned that low dietary protein and a low energy diet reduced phosphorus availability.

Knox and Haramati (1985) stated that the parathyroid hormone plays an important role in renal phosphate re-absorption through stimulation of adenylate cyclase which converts ATP to cAMP that acts as a second messenger, possibly through generation of protein kinase and induced metabolic change, resulting in a decrease of the phosphate transport across the brush border membrane. The authors added that in case of phosphorus depletion, the kidney conserves phosphate and the renal response to the phosphaturic effect of the parathyroid hormone is blunted.

In dairy calves between 9 and 19 weeks of age, **Beighle et al. (1990)** showed that dietary levels of cations and anions had an effect on the concentration of phosphorus in blood, bones and faeces. Diets which had high anions contents, demonstrated a higher concentration of phosphorus in blood and faeces than diets with high cations content and at the same time demonstrated a lower concentration of phosphorus in bones.

Braithwaite (1976) reported that ruminant usually can tolerate a wider dietary calcium and phosphorus ratio without a noticeable reduction in phosphorus availability. Furthermore, **Radostitis et al. (2000)** cited that increasing the intake of calcium might reduce the availability of phytate phosphorus. Moreover, phosphorus deficiency is usually primary under field conditions but might be exacerbated by a deficiency of vitamin D, added excess of calcium, phytic acid, iron, and aluminium and increase magnesium. The authors added that Experimentally large doses of vitamin A might decrease absorption of phosphorus in cattle. Blood level of

phosphorus are not a good indictor of the phosphorus state of an animal because it can remain at normal levels for a long period after cattle have been exposed to a serious deficiency of the element.

2.2.3 Clinical signs of hypophosphatemia

Phosphorus deficiency induces several disease syndromes and affects the function of several systems in different animals. In experimental feeding trials of beef cows on variable amounts of dietary phosphorus, **Call et al. (1986)** recorded that clinical signs of phosphorus deficiency were a general reduction in feed consumption, unthriftness, body weight loss, reluctant to move, bone fracture and impaired reproduction. Moreover, in North Island, **Brooks et al. (1984)** added that clinical signs of phosphorus deficiency a seasonally supplied dairy herd of 90 cows were low milk production, ill-thrift, infertility, pica, lameness and fracture of long bones due to osteodystrophy. Rectal examination of infertile cows revealed small ovaries without palpable follicles or corpora lutea in all age groups.

Forrester et al. (1989) stated that the consequences of hypophosphatemia might include haemolytic anaemia, seizures, altered mentation, cardiomyopathy and skeletal muscle weakness.

Radostitis et al. (2000) recorded that reduction of involuntary intake of food led to retard growth, low milk yield and reduced infertility. Animals have a lag appearance with a narrow chest and small girth, the pelvic is small and the bone is fine and breaks easily. The chest is stab-sided due to weakness of ribs and the hair coat is rough, staring and lacking in pigment. Above mentioned signs are the clinical signs of dairy cows suffering from phosphorus deficiency. In addition, the author added that animals living in a phosphorus deficient area, express additional losses due to botulism.

Ogawa et al. (1987) reported that the clinical signs of phosphorus deficiency in cows are depression, anorexia, pale mucus membranes and reduced ruminal activity. Moreover, **Ogawa et al. (1989)** stated that the signs of phosphorus deficiency occur when serum inorganic phosphorus values decrease to1mg/dl.

In several districts of Sudan, **Ahmed et al. (2002)** observed that native breed heifers with phosphorus deficiency in association with a low zinc and copper content in pasture, soil or animal feed showed delayed puberty, stunted growth and infertility.

In five adult buffaloes from two premises, **Abdou et al. (1986)** recorded that phosphorus deficiency signs were poor milk yield, depraved appetite and stiff gait. These signs were improved after treatment with preparations of organic phosphorus and supplementation with monosodium phosphate.

There exist several reports discussing the relationship between hypophosphatemia and post-parturient hemoglobinuria. **Martinovich and Woodhause (1971)** demonstrated that hypophosphatemia was not always an important cause of post-parturient hemoglobinuria with Heinz body formation in lactating cattle. Then, **Gardner et al. (1976)** found that a high proportion of cows having Heinz-body anaemia were associated with a low level of copper in blood and liver. Moreover, from haematological and biochemical features of 16 cases of post-parturient hemoglobinuria in New Zealand, **Ellison et al. (1986)** concluded that there were two distinct entities of post-parturient hemoglobinuria. One occurs in young cows and is associated with no hypophosphatemia but with Heinz body anaemia, while the other form occurs in multiparous cows and exhibits a low serum inorganic phosphorus level.

In North America, **Macwilliams et al. (1982)** reviewed that post-parturient hemoglobinuria was typified by acute intravascular hemolysis, hemoglobinuria, anaemia and hypophosphatemia especially in high producing dairy cows in their 3^{rd} to 6^{th} lactation.

From vitro experiments, **Wang et al. (1985)** and **Pirzada and Hussain (1998)** concluded that hypophosphatemia caused post-parturient hemoglobinuria by decreasing red cell glycolysis and resultant ATP synthesis. Subnormal concentration of ATP will predispose red cells to alter their structure and function, to a loss or normal deformability and to an increase in fragility and hemolysis with resultant hemoglobinuria. The author showed that the rate of glycolysis was dependent on the inorganic phosphate concentration.

Ogawa et al. (1989) concluded that signs of phosphorus deficiency occurred when serum inorganic phosphorus values decreased to 1mg/ dl and inadequate phosphorus impaired the function and viability of RBCs, producing hemolysis and anaemia.

On the other hand, for post-parturient hemoglobinuria of 11 Holstein –Friesians in 8 dairy herds in East Gippsland, Victoria **Jubb et al. (1990)** suggested that circulating oxidants resulting from hypophosphatemia might cause erythrocyte damage. The disease responded to treatment with anti-oxidants such as methylene blue as well as phosphorus compounds.

In a case of a crossbred cow (Holstein Friesian X Deoni) with post-parturient hemoglobinuria, **Bhinkane et al. (1995)** reported that disease was clinically characterised by coffee coloured urine, anaemia and reduced milk yield and biochemically by a significant hypophosphatemia (2.3mg %). The affected cow responded well to treatment with sodium acid phosphate given 80 g i.v for two days along with supportive therapy for anaemia.

2.2.4 Relationship between hypophosphatemia and fatty liver

Concerning the relationship of phosphorus deficiency and fatty liver, literatures is scanty. **Schulz (1985)** mentioned that post-parturient hypophosphatemia was associated with a increasing fatty infiltration of the liver. Furthermore, from necropsy findings **Radostitis et al. (2000)** concluded that the liver of cows affected with post-parturient hemoglobinuria was swollen and a fatty infiltration degenerative was present.

Some reports state that relationship exist between fatty liver and phosphorus deficiency on the one side and ketosis on the other side. Ketosis was observed more frequently in fat cows than in normally conditioned cows **(Andrews et al., 1991)**. **Gröhn et al. (1983)** found that severely ketotic cows had a higher percentage of fat in their liver than did healthy cows and the extent of fatty infiltration was positively correlated with the concentration of ketone bodies in the blood.

In addition to that, **Reid et al. (1983b)** recorded a higher concentration of 3-hydroxybutyrate in the blood of cows with moderate fatty livers (20%) than in the blood of cows with mild fatty

livers (less than 20% fat). **Oetzel (2001)** classified ketosis into three types and considered fatty liver as a type II ketosis.

For 180 Holstein cows within 40 days after parturition, **Margolles et al. (1988)** reported that hypophosphatemia in 10.7% of cases was associated with hyperacetonemia. **Forrester et al. (1989)** mentioned that severe hypophosphatemia (serum phosphorus concentration below 1mg/dl) was most often associated with diabetic ketoacidosis in small animals. **Jubb et al. (1990)** suggested that hypophosphatemia was likely to be secondary to a metabolic derangement, most probably sub-clinical keto-acidosis in the pre-parturient period caused by sub-maintenance feeding of a heavily pregnant animal.