5 Discussion

The interpretation of field studies is different from properly controlled studies conducted in a laboratory setting. Field studies have the advantage that they are directly related with events occurring in practice and it is also easier to use a large group of cows in a field study. As the results of field studies are not suitable to reveal precise mechanisms but rather are of value to indicate the importance of a disease in principle and giving useful suggestions for the aetiology and consequence of the disease.

Fatty liver is a frequent field problems in high lactating dairy cattle and its diagnosis is very important for veterinarians and owners to make rapid judgement if the cow is either treated or culled to spend time and cost from an economic point of view. The primary objective of this study was to determine suitable methods for diagnosing fatty liver and its economic importance with observations on the relationship between it and hypophosphatemia.

5.1 Liver biopsy technique

Liver biopsy is the most reliable method for diagnosing fatty liver **(Staufenbiel et al., 1993)**. In this study, liver biopsies were obtained from 480 cows without any complication except in one cow that had its pancreas punctured. In a previous study **(Swanson et al., 2000)** it was reported that two calves died either from peritonitis and pneumothorax as a complication of liver biopsy procedures. In contrast, **Harvey et al. (1984)** mentioned that serial liver biopsy procedures do not have any effect on hematologic, histologic or serum biochemical parameters of sheep.

5.2 Estimation of fat content of liver

The results of the present study show that the copper sulphate test for estimation of total lipid content of livers is a reliable method for assessing the degree of fatty infiltration of the liver of dairy cows. The reason for this is apparent in figure 1 which plots the regression of hepatic total lipid measured by the copper sulphate test (y-axis) against results of the gravimetric method (x-

axis) (r=0, 98). The regression lines revealed that there is significant correlation between the two methods. This corresponds to results reported by **Herdt et al. (1983)**, because a strong relationship exists between the fat content of livers and specific gravity. Therefore, the copper sulphate test can be used as field test for estimation of total fat content of livers. Whether the fat content of livers is assessed gravimetrically or by the copper sulphate test depends on the requirements of the work and the technical capabilities of the laboratory.

There exists no previous study on using tissue lysine buffer (ATL) for the preparation of liver biopsies to assess total fat content of livers, rathers **Seigner,(1998)** used ATL buffer for molecular biology to destruct cells and compare different methods for DNA elements' extraction from healthy tissues. This buffer achieved a high percentage of liver cell destruction and consequently enabled accurate measurement of total fat content inside the liver cells.

On the basis of total lipid measurements, **Herdt et al. (1983)** suggested that the liver could be classified into normal, mild, moderate and severe fatty liver when total fat contents were <13, 13-25, 25-34, >34%, respectively. On the same basis, in our study as shown in table 1, the livers were classified into normal, mild, moderate and severe fatty livers when fat contents were ≤15.5, ≤26, ≤33 and >33% respectively. These values were nearly similar to the values reported by **Gaal et al. (1983).**

5.3 Estimation of triglyceride

The recorded triglyceride values of livers in normal, mild, moderate and severe fatty liver (table, 2) were 0-10, >10-15, >15-20, >20 mg% respectively. These values were similar in comparison to values of the same condition recorded by **Muylle et al. (1990).** However they were above levels of liver triglyceride in different fatty liver conditions which were recorded by **Reid and Robert (1983); Gaal et al. (1983)** and **Oikawa et al. (1997).**

Moreover, results of biochemical estimations of total triglyceride, TG/FC and measurements of fat content of livers are considered reliable methods for assessing the degree of fatty infiltration of the liver of dairy cows because of the strong correlation between them. The reason for this apparent in figures 2 and 3 which plot the regression of triglyceride % or TG/FC %, respectively,

against hepatic total lipid %. The established correlations disagree with previous results **(Gaal et al., 1983)** who reported that estimation of hepatic total lipid content is not an acceptable method for assessing fatty infiltration of livers because the high basal level of non-triglyceride lipid masks the increase in hepatic triglyceride content, which is characteristic of fatty livers. In contrast, **Gerloff et al. (1986)** stated that the bovine liver synthesises few fatty acids; triglycerides that accumulate therefore must result from re-esterification of non-esterified fatty acids mobilized from adipose tissue. Fatty liver results from the influx of a large amount of mobilized non-esterified fatty acids.

5.4 Clinical signs

The major clinical signs observed in cows suffering from severe fatty liver were obesity, yellow colour of visible mucus membranes, anorexia, obesity, ketosis, ketonuria and nervous signs. These clinical signs agree with those published previously for the illness **(Reid et al., 1983b; West, 1990; Sevinc et al., 2003).**

Our results revealed that there was positive correlation between body weight and fat content of livers which corresponds with finding reported by **Busato et al. (2002)** who stated that cows in good condition have a greater risk of metabolic problems, because of excessive mobilization of body reserves.

Anorexia observed in cows with severe fatty liver in this study agrees with **Rehage et al. (1996).** Similarly, **West (1990)** reported that feed intake in cows with severe liver damage is low. Anorexia which is characteristic of fatty liver appears to be the result of either metabolic toxaemia due to hepatic failure, a consequence of the resulted from accumulation of triglycerides in liver cells **(Strang et al., 1998; Radostitis et al., 2000),** or due to phosphorus deficiency **(Schulz, 1985).**

Development of icterus can be attributed to an increase in total bilirubin in the blood in case of fatty liver as suggested by **Staufenbiel et al. (1991)** and **Radostitis et al. (2000). West (1990)** stated that the total bilirubin concentration in plasma was a good prognostic indicator of the the degree of liver damage following fatty infiltration. Moreover, the increase in total bilirubin

concentration is likely to be due to hepatic necrosis associated with fatty infiltration, impairing conjugation and excretion of it **(Rehage et al., 1996)**.

Ketosis and ketonuria result from the increase of keton bodies in the blood due to disturbance in the carbohydrate metabolism associated with fatty liver as suggested by **Morrow (1976)** and **Grummer (1993).** Both authors stated that development of fatty liver has a direct effect on the carbohydrate metabolism and, therefore, does influence susceptibility to ketosis.

Nervous signs can be attributed either to hypoglycaemia associated with fatty liver as was observed by **Reid and Roberts (1983)** and **West (1990)** or to failure of the normal hepatic detoxification mechanism reported by **Radostitis et al. (2000).** The reason is that accumulation of triglyceride in the cytoplasm of fatty livers is accompanied by disturbances in hepatic structure and function which may result in hypoglycaemia and in the accumulation of toxic substances like ammonia **(Strang et al., 1998; Zhu et al., 2000)**.

5.5 Relationship between fatty liver and different disease conditions

The relationship between fatty liver and different diseases as shown in table 5 and figure 4 revealed that cows with left displacement of the abomasum suffered from moderate to severe fatty infiltration more frequent than cows of the than other two disease groups (RDA and other diseases). Similar observations have been reported by **Holtenius and Niskanen (1985); Muylle et al. (1990)** and **Rehage et al. (1996).**

The incidence of LDA predominantly in the first weeks of lactation , markedly aggravates the catabolic-metabolic condition Due to the disturbance of the passage digesta during LDA. Feed intake and energy intake decline, consequently resulting in a development of a negative energy balance and fatty liver **(Dirksen, 1967)**.

In addition, an increase in the incidence of endometritis in association with an increase of the fat content of livers was observed as show in figure 5, which agrees with result of **Zerbe et al. (2000)** who stated that a high incidence of endometritis present when fat mobilization is more pronounced. Previous studies **(Morrow et al., 1979; Reid et al., 1983a; Zerbe et al., 2000)**

contained some information about the relationship between an increase of the fat content of livers and depressed immunity that would explain why the high incidence of endometritis is associated withan increase of the fat content of livers. **Wentink et al. (1997)** mentioned that high fat content of liver is related to an impaired immune reaction (mostly cellular immune function). Moreover, an increase in the fat content of livers in the 1st and 2nd week after calving is associated with a decreased functional capacities of polymorphonuclear neutrophilic granulocytes derived from blood and uterus which lead to involved susceptibility of cows to infectious diseases and an increase in the incidence of endometritis **(Zerbe et al., 2000).**

5.6 Relationship between fatty liver and survival rate

Greater percentages (47.4%, 65, 5%) of cows suffering from severe fatty liver either according to the fat contents of liver or triglyceride contents of liver classifications respectively died as shown in table 5. In contrast, cows with mild and moderate fatty livers did not differ in the mortality rate from cattle with normal livers, this finding is similar to results reported by **Herdt et al. (1983)** and **Gerloff et al. (1986)** indicating that the disease rate in cows with severe fatty infiltration tended to be greater than in other fatty infiltration groups. This corresponds to observations of **Gerloff et al. (1986)** who reported that the highest mortality rate occurs in cows with severe depositions of fat in livers. On the other hand, **Reid and Roberts (1983)** contented that mild and moderate fatty liver is rather a sub-clinical entity.

In cases of severe fatty liver, the accumulation of fat in livers leads to the impairment of functions of the liver and to development of liver failure **(Herdt et al., 1982; Reid, 1986; West, 1990)**. Experimental studies also showed that an increase in the fat content of livers leads to a reduction of ureagenesis and an increase in the concentration of ammonia in the blood which is a toxic product and plays a role towards morbidity associated with severe hepatic lipidosis **(Strang et al., 1998; Zhu et al., 2000)**. In addition, severe hepatic lipidosis is associated with reduced immune competence and affected cows have a low resistance to diseases **(Wentink et al., 1997; Zerbe et al., 2000)**. Mild and moderate fatty liver may be associated with an decrease in the protein synthetic capacity without resulting in changes in the general clinical condition **(Reid and Collins, 1980)**.

5.7 Clinical chemistry

Regarding the relationship between fat content of livers and serum chemistry as shown in table 8, it was observed that the mean serum phosphorus concentration decreases with an increase in the fat content of livers. These results are similar to those reported by **Schulze (1985)**. Fatty liver associated with hypophosphatemia is attributed to a decrease in food intake during hypophosphatemia **(Call et al., 1986; Fishwick and Hemingway, 1989; Goff, 2000)** leading to a reduction of energy intake and excessive fat mobilization which accumulates in the liver, developing fatty liver **(Herdt, 1988; Grummer et al., 1990; Wentink et al., 1992)**.

On the other hand, fatty liver leads to hypophoshatemia either through a decreasing its dietary intake **(Forrester et al., 1989)** or due to ketoacidosis, developed during pathogenesis of fatty liver **(Knochel, 1977)**

Moreover, a negative correlation between serum concentration of potassium and fat contents of livers was established. No reports about this relationship do exist but hypokalaemia associated with fatty liver may be referred to a decrease in food intake which accompanies fatty liver **(Smith, 1996; Radostitis et al., 2000)**.

Decreases in the urea concentration associated with an increase in fat contents of livers may suggest that accumulation of fat in the livers leads to failure of livers which affects the ureagenesis process, resulting in a decrease in the urea concentration in blood and to an increase in ammonia. This process is consistent with observations in vitro **(Strang et al., 1998; Zhu et al., 2000)**.

Many authors prefer to measure enzyme activities and consider them to be more sensitive and specific to assess the liver function **(Rehage et al., 1996; Pechova et al., 1997; Komatsu et al., 2002)**. In our study, it was observed that only ASAT showed a significant increase in correlation with an increase in total fat content of livers. On the other hand, GLDH measurements had no relationship with lipid contents of livers. This coincides with results reported by **Gerloff and Herdt (1984)** and **Schäfer et al. (1991)**. According to our results, the activity of ASAT yields the

best evidence for the deposition of fat in livers because lipid infiltration present in muscles and livers are so high that ASAT activity my reflect muscle and hepatic damage **(Reid et al., 1983b)**.

Increases in the serum concentration of total bilirubin in association with increasing fat contents of livers were similar to **Reid and Roberts (1983)** and **Pechova et al. (1997)**. An elevation of total bilirubin may be attributed to hepatic cell degeneration resulting from an accumulation of fat in hepatocytes **(Radostitis et al., 2000)**.

5.8 Hematology

Referring to the blood picture, the results of this study revealed significant correlations though weak with most blood parameters this may be attributed to the fact that there exist multiple several factors affecting this correlation, especially, in the diseased cows in this study were recorded.

All cows included in this study, irrespective of their fat content, showed a negative correlation with the total number of total leucocytes, this correlation was weak but significant. Such relationship already was observed by **Hussain (1989), Cai et al. (1994)** and **Da Silva et al. (1998)**. A general suppression of total blood leucocyte numbers is suggested to be caused by disturbed carbohydrate/lipid metabolism **(Morrow et al., 1979; Reid et al., 1983a)**. Moreover, decreased total leucocyte counts in case of high fat content of livers is discussed with an increase prevalence of endometritis which is associated with severe fatty liver as mentioned before. The reason for the increased lymphocytes in this study is unknown.

The blood indices in this study revealed a development of macrocytic normochromic anaemia in association with an increase in fat contents of livers. Macrocytic normochromic anaemia is due to cobalt deficiency **(Jain, 1986)**. **Uvlund (1990)** and **Dirksen et al. (2002)** suggested that relationships exist between fatty liver and cobalt deficiency in sheep and cattle, and consequently with vitamin B12 deficiency. In this study, we cannot completely confirm this relationship because the cows included in this study were clinical diseased cows, this result needs further investigation.

5.9 Histopathology

The histopthological picture in the present studies confirms earlier reports **(Reid and Collins, 1980; West, 1990)**, who reported that excessive deposits of fat occur in livers during fatty liver syndrome. The histological classification of increased hepatic lipids in this study is in agreement with classification of fatty livers by biochemical analysis of the liver fat content **(Gaal et al., 1983)**.

West (1990) found that lipids are diffusely distributed in the liver and suggested that its location was centrilobular, midzonal or covering the entire lobule according to the severity of fatty infiltration of livers. In the present study, it was revealed that in cases of mild and moderate fatty livers, the accumulation of fat was associated with the hepatic structural unit. Zones centred around the afferent vascular vessels were progressively involved.

The proximity to the afferent blood supply may be of importance in case of metabolic disturbance in mammals. Fat depots brought into the liver in case of starvation show up firstly in the cells nearest to the portal unit structure and their branches **(Schiff, 1965)**. In severe cases, entire lobules are affected with large amounts of diffusely distributed hepatic lipids. It was impossible to identify a zonal pattern of deposition which agrees with **Reid and Collins (1980)**.

5.10 Ultrasonographic examination of fatty liver

In evaluations of fatty infiltrations of livers in dairy cattle, ultrasonography can be used as a non-invasive method of diagnosis. Changes in the liver parenchyma usually give rise to various ultrasonographic patterns. In general, neoplasms result in a hypoechoic area while chronic inflammatory processes result in a hyperechoic image **(Taylor and Milan, 1976)**. Fatty changes in the liver, in particular, cause specific changes in the ultrasonograms because of the high echogenicity of fat. The presence of fat tissue and masses of fat can be demonstrated by ultrasound **(Behan and Kazam, 1978)**. The high echogenicity of fat is due to its lower acoustic impedance (1.38 Rayl x 10^{-5}) in contrast to that of normal liver tissue (1.65 Rayl x 10^{-5}) **(Sanders and Everette, 1980)**.

B-mode ultrasonograms of fatty infiltration of the liver in cattle are usually characterized by an increased parenchymal echo (bright pattern) and a decreased echo penetration at deeper areas of the hepatic tissue (deep attenuation) **(Braun et al., 1996)**. These high amplitude echoes are seen evenly distributed throughout the liver parenchyma **(Joseph et al., 1979)**. The sound velocity and attenuation coefficient were found to be higher in fatty livers than in normal livers **(Nishimura et al., 1986)**. In addition, hepatic and portal veins are not usually visible (vascular blurring) in case of severe fatty infiltration of livers **(Acorda et al., 1994a)**.

In this study, the use of ultrasonography showed actual hepatic changes in cows, severe fatty infiltration of livers resulted in increased echogenicity (bright pattern) and lower deep attenuation (dark pattern). This image was a useful basis for the diagnosis of fatty infiltration and it agrees with the image described in humans **(Mizuguchi et al., 1986)** and dairy cattle **(Acorda et al., 1995)**.

No study yet exists on the measurement of portal vein diameters and on the size of the gall bladder in fatty livers. In our study, the results revealed that there was significant negative correlation between portal vein diameters and fat contents of livers (picture 8). Because cows suffering from severe fatty livers become anorexic this leads to a decrease in the blood flow in mesenteric blood vessels and consequently to a low blood volume in the portal vein and a decrease in its diameter **(Braun and Gerber, 1996)**. In contrast, the size of the gall bladder increased with increasing total fat content of livers (picture 9). This may be due to bile retention in the gall bladder in case of anorectic fatty liver cows **(Radostitis et al., 2000)**.

Based on the evidence described in this study, fatty livers constitute great problems in dairy cattle management and causes several economic losses which need further investigation, e.g. an the effect of seasons on the incidence of fatty liver. Moreover, effects of the fatty liver syndrome on milk production are needed to detect the dominant interaction between different management systems and fatty cow syndrome.