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BIOCHEMICAL EVALUATION OF DOWNER DAIRY COWS WITH FATTY LIVER AND ESTIMATE PROGNOSIS OF SURVIVAL RATE IN IRAN

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ABSTRACT

This study evaluated the relationship between severity of fatty liver and macromineral status in downer dairy cows with fatty liver and determined the usefulness of selected liver biochemical analyses for assessing prognosis. Blood samples were collected from 30 Holstein downer cows that could not rise 24 hours after recumbency and after first treatments. Serum activity of muscle and liver-derived enzymes and concentration of non-esterified fatty acids (NEFA), cholesterol and macrominerals (Ca, Mg, K, Na, P) were determined. Serum concentration of NEFA and cholesterol, and NEFA/cholesterol ratio are good indicators of fatty liver. In this group of 30 downer cows, 70% had calved within first week and 30% calved within first 100 days of sampling. In this study about 70% of dairy cows had biochemical evidence from some degree of fatty liver that 20% were with mild and 50% were with moderate fatty liver and 80% from they were in the 1st week after calving. The 50% downer cows were with moderate fatty liver that all of them culled and had significantly higher NEFA/cholesterol ratio. The NEFA/cholesterol ratios herein were about 2 times higher in cows with moderate fatty liver compared with the reference cows. The prognosis is guarded for downer cows with moderate fatty liver.

Keywords: Downer Cow Syndrome, Milk Fever, Fatty Liver, Non-Esterified Fatty Acids, Cholesterol, Dairy Herds

INTRODUCTION

Downer cow syndrome is a complication of recumbency associated with milk fever (Radostits *et al.*, 2007). The syndrome occurs mainly in the early post parturient period and is caused by several diseases. Downer cow syndrome refers to cows that become recumbent and fail to rise; this is a major concern in dairy farms worldwide. The most common cause of downer cow syndrome is hypocalcemia (milk fever) (Barrington, 1998; Cox, 1988) but it is also caused by injuries, muscle damage, macro mineral deficiencies, toxic mastitis or metritis (Gerloff, 2001). Approximately 58% occurred within 1 day of parturition and 37% occurred during the first 100 days of lactation (Radostits *et al.*, 2007). Hepatic dysfunction particularly fatty liver may also contribute to cows becoming downers (Rukkamsuk *et al.*, 1999). Almost all high producing dairy cows are in negative energy balance in early lactation because energy requirements exceed feed consumption capacity (Collins and Reid, 1980). The liver plays a central role in metabolism and dairy cows are generally prone to liver disease (Staufenbiel *et al.*, 1993); a high proportion of dairy cows experience fatty liver before and after parturition (Rehage *et al.*, 1999; Reid, 1980).

Although fatty liver is an important risk factor for the occurrence of downer cow syndrome (Rukkamsuk *et al.*, 1999), it is often misidentified or overlooked because it is difficult to diagnose. Clinical signs in downer cows usually do not accurately reflect hepatic dysfunction, unless the liver damage is severe. There are a variety of liver biochemical parameters, such as aspartate aminotransferase (AST), alkaline phosphatase (ALKP), gamma glutamyltransferase (GGT), cholesterol(CHOL), non-esterified fatty acids (NEFA) and lactate dehydrogenase (LDH), which provide some diagnostic information (Bobe *et al.*, 2004). There have been few reports of the relationship between downer cow syndrome and fatty liver.

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The purposes of the study were to evaluate of liver enzyme activity, serum macromineral status, and other biochemical analytes in culled downer cows.

MATERIALS AND METHODS

Animal Selection

Thirty Holstein cows (6 of which were first-calf heifers) from 5 industrial dairy farms in Iran were used. Cows that became recumbent were eligible for inclusion in the study and were referred by the local veterinarians who serviced the herds. All cows were sampled 24 hours after recumbency and after first treatments were administered. Downer cows in the present study had failed to rise within 24 h after the 1st treatment.

Data collected when the cows were examined included age, parity, date of calving, recent health and production problems, time (h) from the onset of recumbency, and vital signs. A thorough physical examination was performed on each cow including rectal temperature, pulse rate, inspection of mucous membranes and examination for mastitis, metritis and bone fractures. Then blood samples were collected. Cows without signs of disease other than hypocalcemia were then treated with 500 mL of calcium-magnesium- phosphorous solution (CMP) IV, 250 mL of 40% calcium borogluconate SC, 20 mL of phosphorous IV, 500 mL of 50% dextrose IV, and 250 mL propylene glycol PO.

The 30 cows that were finally included in the study (from a total of 150 initially referred) fit the definition of “downer cows.” These cows were between 1 and 90days after calving. None of them had a history of musculoskeletal injury, nor showed evidence of other disease (such as fever, vaginal discharge, mastitis).

Sample Collection

Blood samples were collected from the jugular vein of each cow using an 18-gauge needle into glass tubes without anticoagulant. After clotting for 30-45 min, serum was separated at the farm by centrifugation at $1600 \times g$ for 15 min, transferred to plastic vials, and transported at 4°C to the laboratory, where it was stored frozen at -20°C. Frozen serum was analyzed for aspartate aminotransferase (AST), alkaline phosphatase (ALKP), gamma glutamyl transferase (GGT), cholesterol (CHOL), creatine phospho kinase (CPK) non-esterified fatty acids (NEFA) and lactate dehydrogenase (LDH), calcium and phosphorus concentrations.

Serum Biochemical Analysis

Spectrophotometric kinetic methods were used to determine serum activities of aspartate aminotransferase (AST) (Thefeld *et al.*, 1974), alkaline phosphatase (ALKP) (Schlebusch *et al.*, 1974), gamma glutamyltransferase (GGT) (Szasz, 1976), and creatine phospho kinase (CPK) (Horder *et al.*, 1991). All measurements were obtained at a temperature of 30°C. Colorimetric spectrophotometric methods were used for determination of cholesterol (CHOL) (Roeschlau *et al.*, 1974) concentrations. Commercial kits were used for determination non-esterified fatty acids (NEFA) (Wako Cod 999-75406; Wako Chemicals GmbH, Neuss, Germany) concentrations. All blood samples were analyzed for serum total Calcium (Ca) concentrations by flame atomic absorption spectrophotometry. Total serum concentration of phosphorus (P) was determined using the heteropoly acid-blue method (Boltz and Lueck, 1958).

Statistical Analysis

Analysis was performed using a commercial software program (SPSS, version 16.0; SPSS, Chicago, Illinois, USA). The Pearsonrank bivariate correlation was used to investigate the relationship between variables. Finally, a Paired Samples T test was used to compare medians of biochemical parameter measurements in the 2 different clinical outcome groups (normal— abnormal). For all tests, values of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Results

Clinical Outcome

The serum biochemical results of these 30 downer cows are presented in Table 1.

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Table 1: Blood and liver parameters test results from normal downer cows and downer cows with mild and moderate fatty liver

Parameter	AST (U/L) Mean	ALP (U/L) Mean	GGT (U/L) Mean	CHOL (mg/dl) Mean	NEFA (mmol/L) Mean	NEFA/CHOL Mean	Ca (mg/dl) Mean	P (mg/dl) Mean	LDH (U/L) Mean	CPK (U/L) Mean
Normal n=9	72.33	99.75	27.75	186.33	0.38	0.072	10.43	6.64	1018.76	110
Mild fatty liver n=6	265	103.5	24.5	134.3	0.87	0.24	7.13	4.76	4134.6	853.83
Moderate fatty liver n=15	220.8	141.2	36.6	101.47	0.85	0.32	8.08	5.02	2475	1059.5

AST — aspartate aminotransferase, ALP— alkaline phosphatase, GGT — γ -glutamyltransferase, CHOL— cholesterol, NEFA — non-esterified fatty acids, Ca — calcium, P — phosphorus, LDH — lactate dehydrogenase, CPK — creatine phospho kinase.

Classification of cows according to the clinical and biochemical outcome and the severity of fatty liver is shown in Figure 1. The time frame for their recovery was between 1 and 6 d after 1st treatment. The 21 cows (70%) could not rise and culled by 7 days after 1st treatment that the 15 cows had moderate fatty liver and the 3 cows had mild fatty liver.

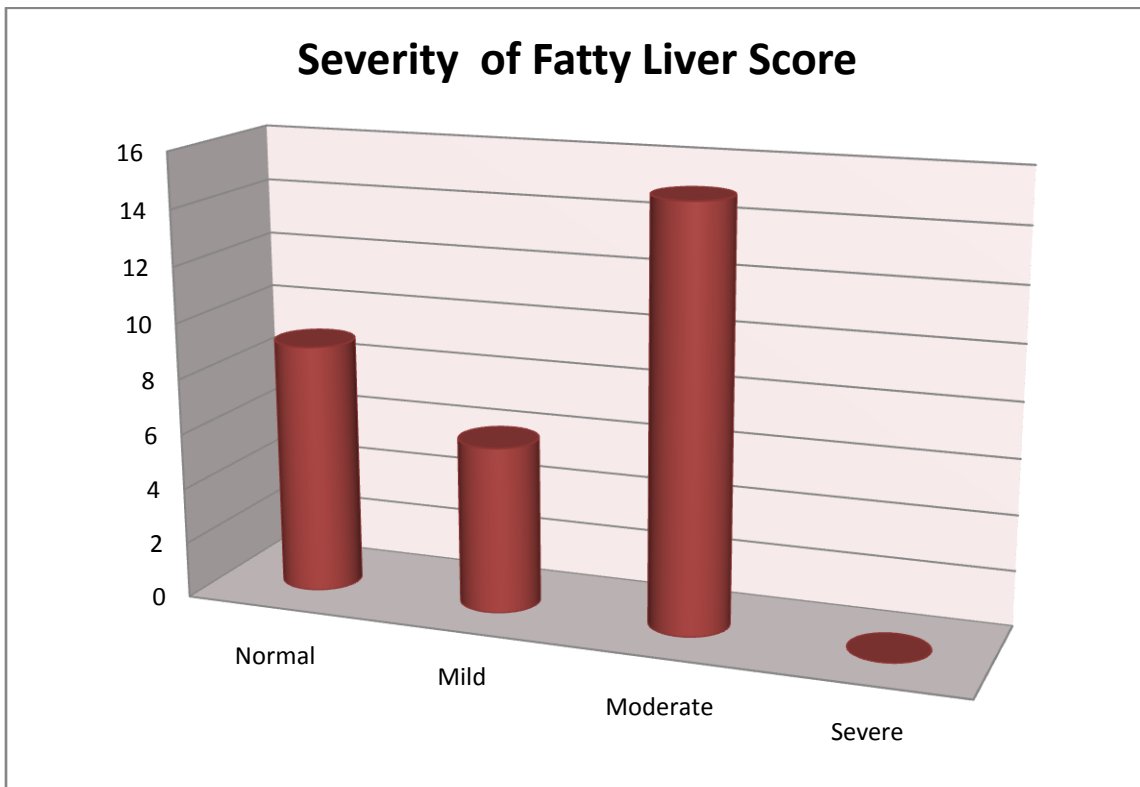


Figure 1

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For 9 parameters (LDH, AST, ALKP, CPK, GGT, CHOL, Ca, P, and NEFA) there were significantly different parameter distribution medians in animals that were normal versus abnormal (Table 2).

Table 2: Median values of the parameters with statistically significantly different means ($P < 0.05$) between the two outcome groups

Parameter	AST(U/L) Med	ALP(U/L) Med	GGT(U/L) Med	CHOL(mg/dl) Med	NEFA(mol/L) Med	NEFA/CHOL Med	Ca(mg/dl) Med	P(mg/dl) Med	LDH(U/L) Med	CPK(U/L) Med
Normal	69	102	25	142.50	0.48	0.05	10.50	6.60	1257	87
Abnormal	281.50	154	49	74.50	0.84	0.31	7.05	4.8	3151	1568.50

AST — aspartate aminotranferase, ALKP— alkaline phosphatase, GGT — γ -glutamyltransferase, CHOL— cholesterol, NEFA — non-esterified fatty acids, Ca — calcium, P — phosphorus, LDH — lactate dehydrogenase, CPK—creatineposphokinas

Distribution of downer cows for purposes of different parameters has been shown in Figure 2, Figure 3, Figure 4, Figure 5 and Figure 6.

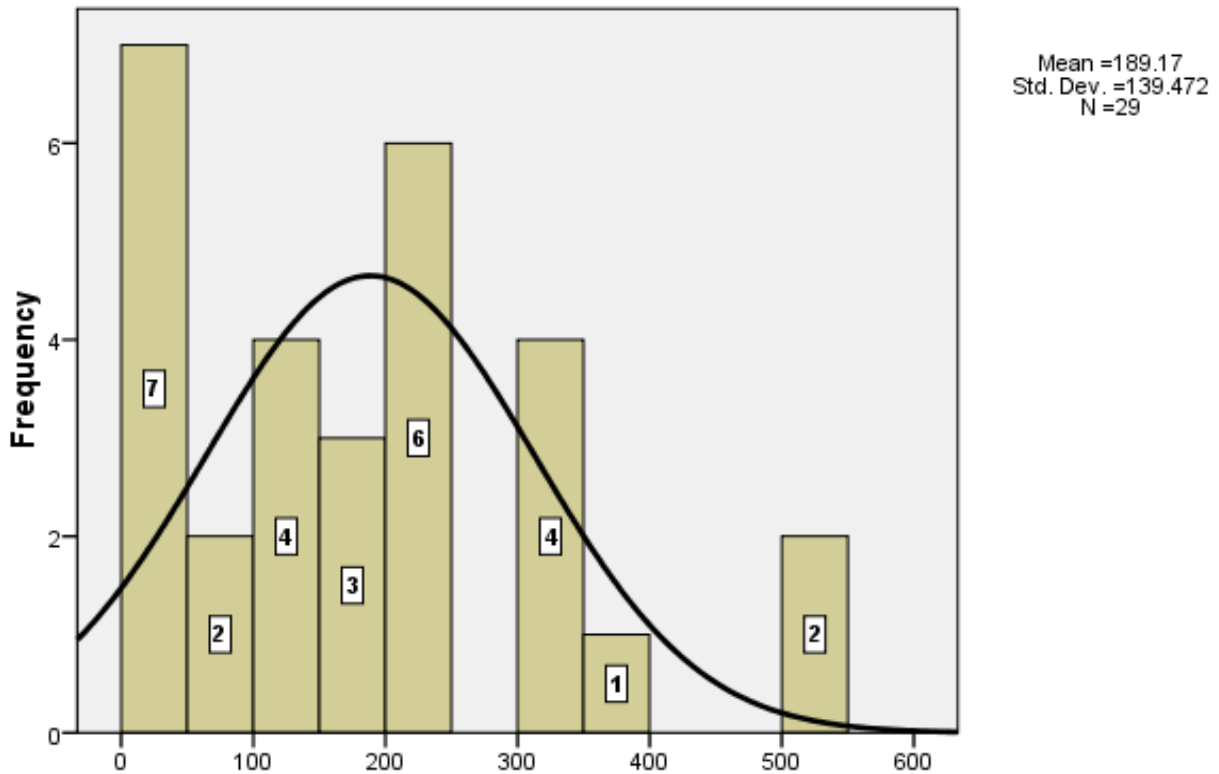


Figure 2: AST histogram with normal curve

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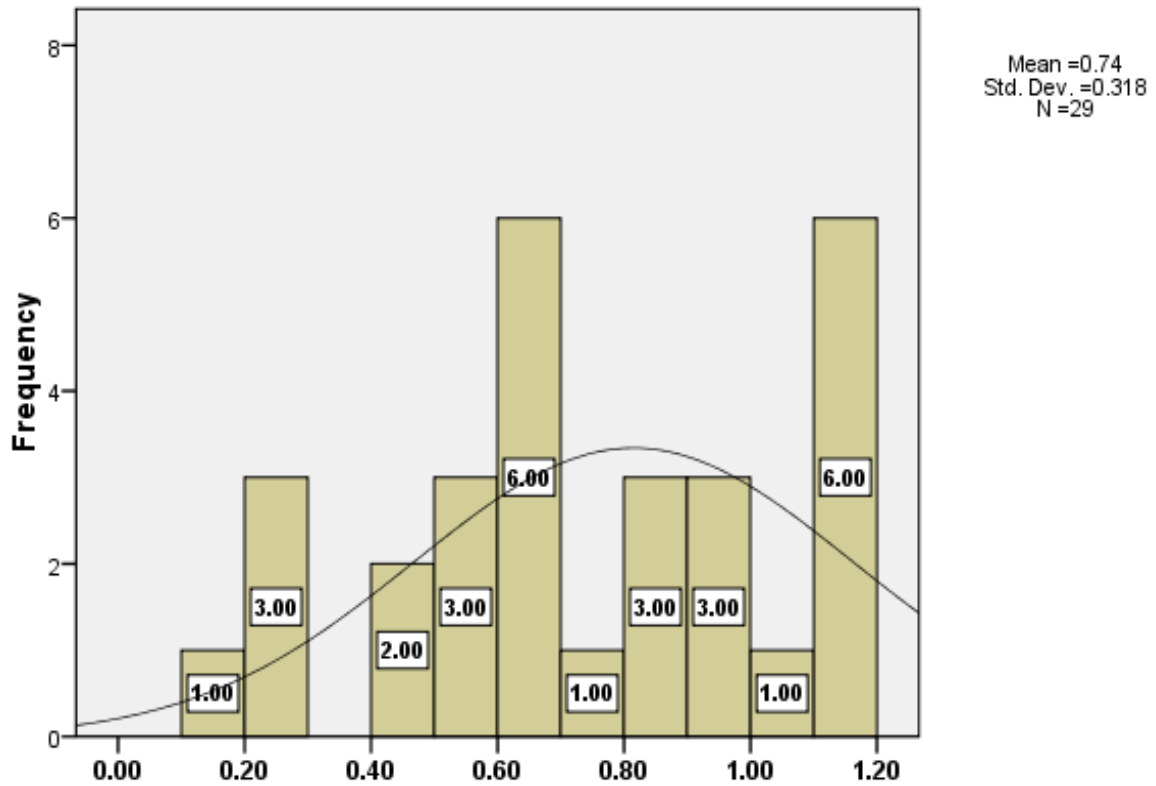


Figure 3: NEFA histogram with normal curve

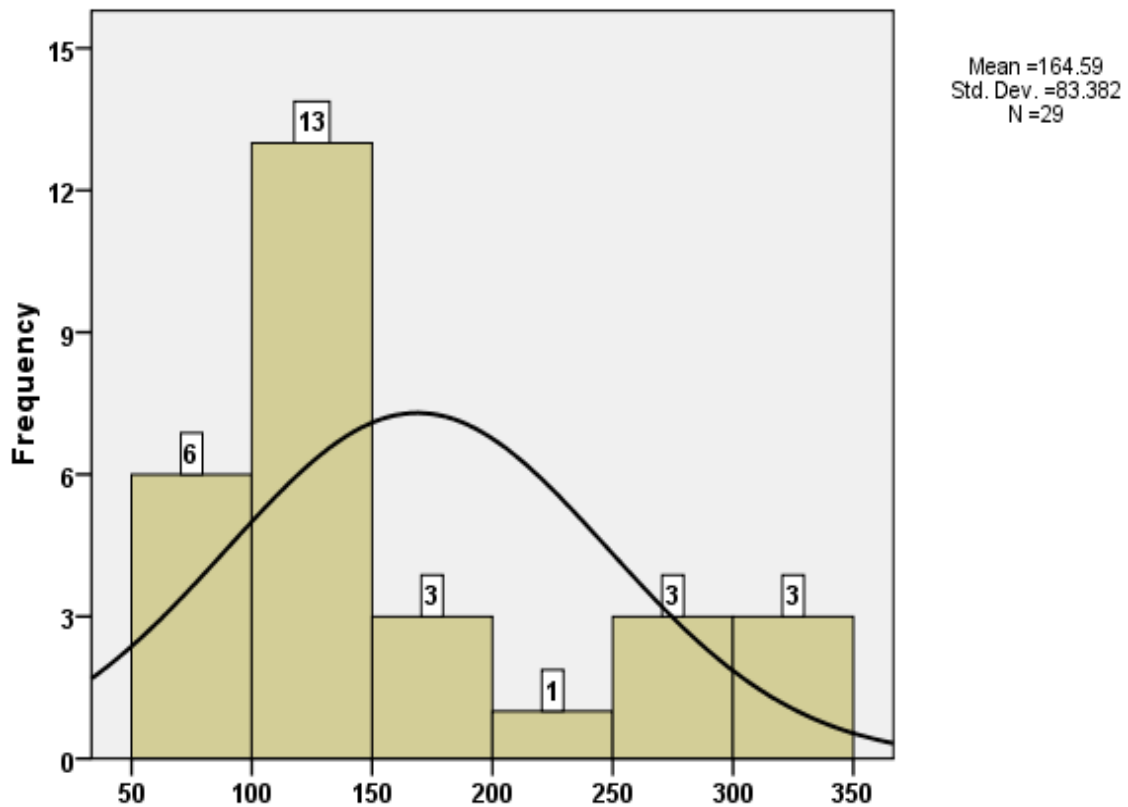


Figure 4: Cholesterol histogram with normal curve

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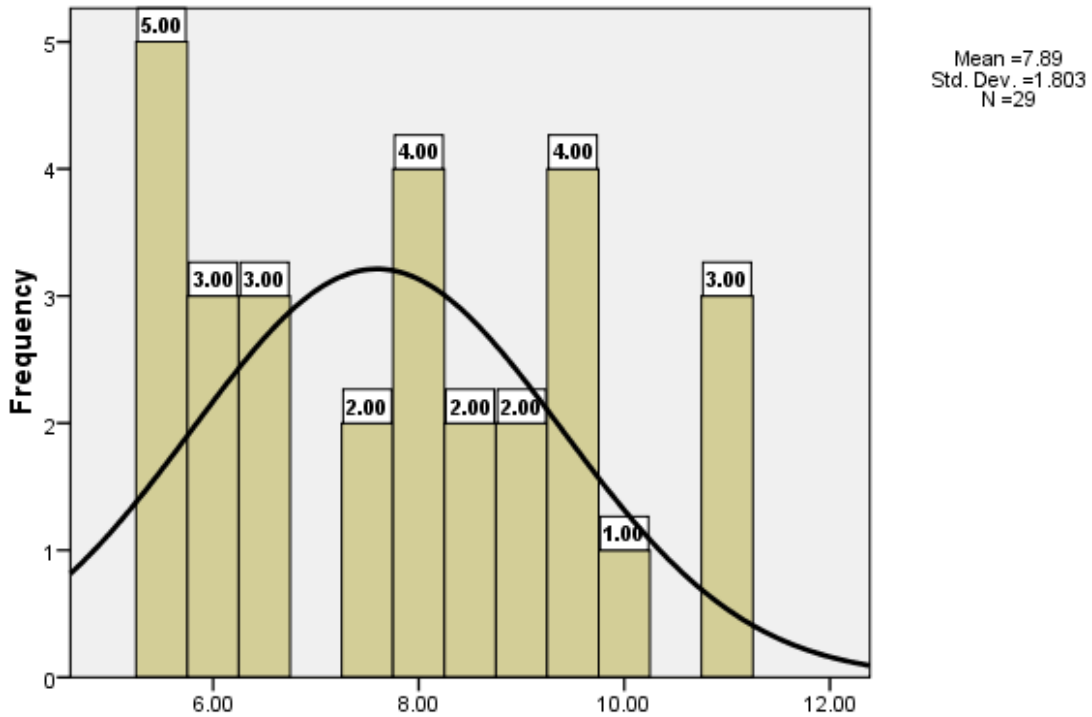


Figure 5: Ca histogram with normal curve

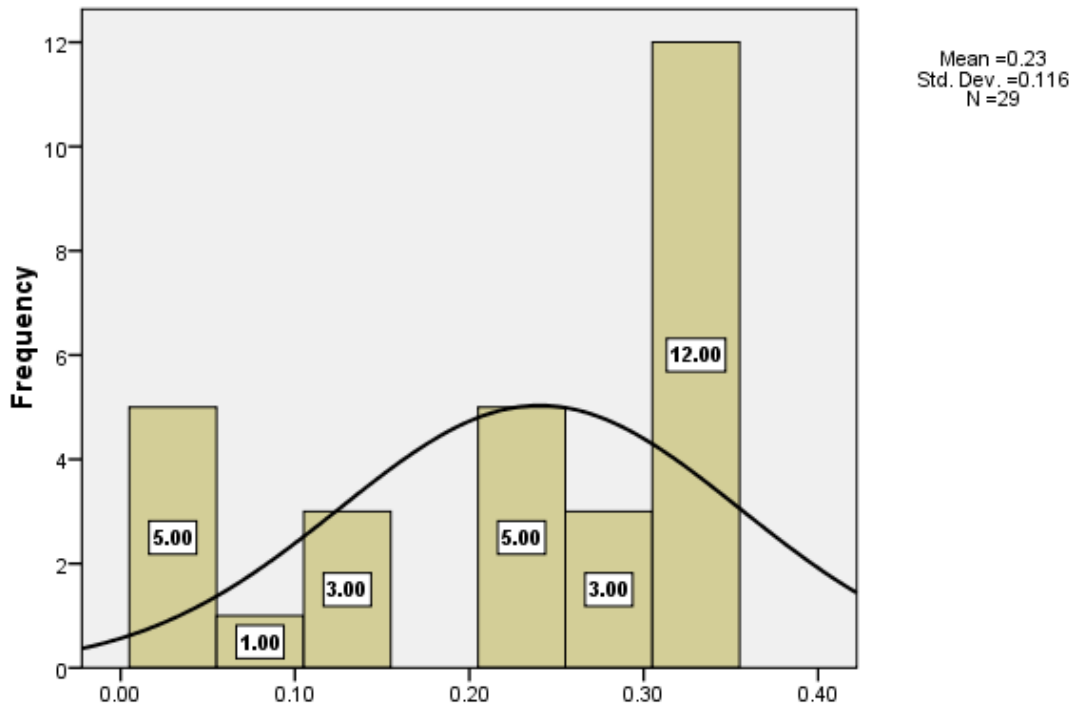


Figure 6: NEFA/Cholesterol histogram with normal curve

Serum Biochemical Analyses

Serum CPK activity was very high in 80% of the downer cows, and mean CPK activity increased significantly from the mild to moderate fatty liver groups (Table 1) and was significantly correlated with parameters such as NEFA, Cholesterol and Ca (Table 3).

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Mean serum NEFA/Cholesterol increased significantly with the severity of fatty liver (Table 1) and was significantly correlated with parameters such as CPK and Ca (Table 3).

Mean serum AST activity was significantly higher in downer cows with mild and moderate fatty liver (Table 1) and was significantly correlated with parameters such as ALKP and parity (Table3).

Downer cows had significantly lower mean serum Ca and P concentrations, but these means were not significantly different between the fatty liver groups (Table 1).

Table 3: Pearson correlation coefficients (r) for selected blood and liver variables, from downer cows. All correlations presented are significant (P< 0.05)

	AST	ALK P	CPK	LDH	GG T	CHO L	NEF A	Ca	P	NEF A/CH OL	Parity	Culli ng days after partu ration
AST		0.439										- 0.569
ALK P	0.439			- 0.456								
CPK						0.429	- 0.637	- 0.527		- 0.507		
LDH		- 0.456										- 0.394
GGT									0.495			
CHO L			0.429				- 0.428		- 0.608	- 0.744		0.468
NEF A			- 0.637				- 0.428		0.536	0.480		
Ca			- 0.527				0.536					
P					0.49 5	- 0.608						
NEF A/CH OL			- 0.507			- 0.744	0.480					
Parity	- 0.569			- 0.394								
Culli ng days after partur tion						0.468						

Median serum AST, ALKP, GGT, NEFA and LDH concentration was significantly elevated in mild and moderate fatty liver groups (Table2). Mean serum NEFA concentration was significantly higher in cows with mild and moderate fatty liver compared with normal downer cows (Table 1). The NEFA/cholesterol ratios averaged 0.07 in the reference cows, 0.24 in the mild fatty liver group, rose to 0.32 for the moderate fatty liver group (Table 1).

Cholesterol was significantly correlated with P, NEFA and culling days after parturation (Table 3).

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Discussion

One of the aims of the study was to investigate the occurrence and severity of fatty liver in downer cows. In this group of 30 downer cows, 70% had calved within first week and 30% calved within first 100 days of sampling. About 70% of dairy cows had biochemical evidence from some degree of fatty liver that 20% with mild and 50% with moderate fatty liver and 80% from they were in the 1st week after calving (Reid, 1980). This finding supports the theory that fatty liver is present prepartum (West, 1989) and is likely caused by mobilization of body reserves just before calving (Top *et al.*, 2005).

The downer cows had significantly lower median serum Ca concentration compared with the reference and healthy fresh cows. This was expected, because hypocalcemia is the most frequent cause of recumbency in fresh cows (Barrington, 1998). In this study 40% of downer cows were hypocalcemic (Ca concentrations below 7.2 mg/dl) despite had been treated for hypocalcemia. The 23 cows of the downer cows suffered severe hypocalcemia (Ca concentrations below 6.5 mg/dl). There was no difference in serum Ca between the fatty liver groups.

The Ca/P ratio were abnormal in 21 downer cows (about 70% of cases).

First-lactation animals accounted for 20% of the downer cows enrolled in this study. Three of these 6 animals had mild fatty liver and three others had moderate fatty liver, suggesting that heifers are also vulnerable to this condition. This result is in agreement with some studies (Cebra *et al.*, 1997) but in contrast to others (Gerloff, 2001).

Fatty liver is related to post-parturient disorders like hypocalcemia, ketosis, metritis, which occur more often in older cows. It has been reported to be more common in cows than in heifers (Cox, 1988). In this study only 40% of downer cows with moderate fatty liver had 6 gestation and above. Also, the negative energy balance, which typically follows the higher milk production of dairy cows after their 2nd lactation, increases the risk for fatty liver in high producing multiparous cows compared with heifers.

The lack of postmortem examinations on the other cows is a limitation of the study, as the cause of death could not be verified.

Serum AST activity may have value in diagnosing fatty liver (Reid *et al.*, 1983). Others (Szanszlo and Karsai, 1991; Johannsen *et al.*, 1993) have raised doubt about the value of AST, mainly because it is not liver-specific (Garry *et al.*, 1994) and is easily elevated in muscle damage (Kalaitzakis *et al.*, 2010). In downer cows with increased AST activity, concurrent analysis of serum CK activity helps to identify the origin of AST (muscle or liver). In the present study, increases in AST were likely due to muscle damage, because the correlation between serum CK and AST activity was high. The diagnostic value of AST in downer cows suspected for liver dysfunction is diminished.

Fatty liver is characterized by abnormal lipid and lipoprotein concentrations (Rayssiguier *et al.*, 1988).

In the present study, serum cholesterol concentration was significantly decreased in cattle with moderate fatty liver compared to the healthy cows and cows with mild fatty liver, and was inversely related to NEFA concentrations. These results are in accordance with earlier reports in which fatty liver infiltration was associated with decreased serum cholesterol, higher NEFA, and higher NEFA/cholesterol ratio (Holtenius, 1989). The NEFA/cholesterol ratios herein were about 2 times higher in cows with moderate fatty liver compared with the reference cows. Ratios of NEFA/cholesterol > 0.3 in downer cows suggested that at least moderate fatty liver was present.

In the present study, serum NEFA concentration was increased in cows with moderate fatty liver, an observation which agrees with earlier published information (Zerbe *et al.*, 2000). Non-esterified fatty acids are considered useful for detection of fatty liver in downer cows, as a high NEFA concentration is indicative of extended lipid mobilization and is highly correlated with liver lipid content (Szanszlo and Karsai, 1991). Serum NEFA gradually increases during the last week before parturition and then acutely increases at calving, which triggers even more fatty liver infiltration (Vazquez-Añon *et al.*, 1994). In downer cows this phenomenon is more intense because the appetite loss (Dale *et al.*, 1979) and the difficulties in accessing food lead to a higher negative energy balance, which in turn increases NEFA mobilization and blood concentration. The NEFA serum concentration is also stress-sensitive (Kauppinen, 1983), which increases NEFA release, resulting in more rapid lipid accumulation in the liver.

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The prognosis of downer cows with moderate fatty liver was poor. We speculate that the coexistence of fatty liver with hypocalcemia may have negatively affected the downer cows and worsened their prognosis.

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Research Article

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