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The Clinical-Chemical Parameters, Serum Lipoproteins and Fatty Infiltration of the Liver in Ketotic Cows

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Abstract: The objective of this study was to provide a unique insight into the relationship between ketosis and fat cow syndrome using clinical-chemical parameters, serum lipoproteins and histopathological changes of the liver. The cholesterol and protein concentrations were significantly lower in the ketotic cows ($p<0.05$) than in the controls. The glucose and HDL-cholesterol levels were significantly lower in the ketotic cows ($p<0.01$) than controls.The total bilirubin, CPK levels ($p<0.05$), and direct bilirubin, albumin levels ($p<0.01$) were significantly higher in the than controls. The percentage of fatty in filtration of the liver parenchyma varied from 27 to 42 percent in seven ketotic caws and was 13 per cent in one ketotic cow. In light of this a relationship was established between postpartum clinical ketosis and fat cow syndrome.

Key Words: Lipoprotein, Fat cow syndrome, Ketotic cows.

Ketozisli Sığırlarda Klinik-Kimyasal Parametreler, Serum Lipoproteinleri ve Karaciğer Yağ İnfiltrasyonu

Özet: Sunulan çalışmanın amacı, ketozisli sığırlarda klinik-kimyasal parametreler, serum lipoproteinleri ve karaciğer yağ infiltrasyonunun belirlenmesi ile, hastalığın yağlı karaciğer sendromu ile ilişkisine ışık tutmaktır. Ketozisli sığırlarda kolesterol ve protein konsantrasyonları kontrol grubu hayvanlara göre ($p<0.05$) düzeyinde, glikoz ve HDL-kolesterol konsantrasyonları da ($p<0.05$) düzeyinde, direkt bilirubin ve albumin konsantrasyonları da ($p<0.01$) düzeyinde yüksek bulundu. Karaciğerin histopatolojik muayenesinde 7 sığırda %27-42 arasında şiddetli, bir sığırda ise %13 düzeyinde orta şiddetli karaciğer yağlanmasına rastlandı. Buna göre, doğum sonrası şekillenen klinik ketozis karaciğer yağlanması ile önemli ölçüde ilişkilidir.

Anahtar Sözcükler: Lipoprotein, karaciğer yağlanması, ketozisli sığır

Introduction

Many-high-yielding dairy cows have a negative energy balance during the first few weeks of lactation. The demand of the mammary gland for glucose often is greater than the glucose available. This imbalance increases fat mobilization, and increases hepatic ketogenesis. An increase in keton-body concentration during early lactation indicates general fat mobilization. Hyperketonemia and fatty infiltration of the liver become particularly pronounced during ketosis (1-4).

Bovine ketosis is a common and economically significant disease, and may be primary or secondary subclinical. Primary ketosis generally occurs in housed cattle and is seen ten days to six weeks after parturition, with maximum incidence at three weeks (4, 5).

Fatty liver syndrome is a common problem due to a generalised mobilization of fat. Fatty infiltration of the liver occurs as the result of energy requirement during early lactation, particularly of high-yielding cows, outstripping the energy supplied by the feed, so that body reserves have to be mobilized. There is a rapid loss of body weight and intracellular fat accumulates in organs such as the liver. This produces a syndrome in which the affected cattle are more susceptible to metabolic, infectious and reproductive problems (2, 4). Fat mobilization is closely associated with high incidence of severe ketosis during early lactation (6). A fatty liver has been a common finding in severe cases of bovine ketosis (5).

The diagnosis of fat cow syndrome and ketosis is most often based on history, physical findings, and failure to respond promptly to what seems to be appropriate therapy. Laboratory methods are available which can aid the practitioner in accurately assessing the degree of liver lipidosis and lost function. These include non-specific enzymatic tests, specific enzymatic tests, quantitative assays of hepatic lipid content, and function tests of the

liver itself (7). The function tests of the liver are too cumbersome to be carried out under field conditions and liver biopsies are seldom permitted by herd owners. If the possible influence of bovine ketosis on the liver is investigated under field conditions it is practical to measure the plasma activities of the liver-specific enzymes and the plasma concentration of the total bilirubin and urea (8). The determination of serum lipoprotein in dairy cows during early lactation would seem to be of interest both for understanding the pathogenesis of fatty liver syndrome and diagnostic purposes (9).

The objective of this study was to provide a unique insight into the relationship between ketosis and fat cow syndrome using clinic-chemical parameters, serum lipoproteins and histopathological changes of the liver.

Materials and Methods

Animals:

Sixteen Holstein cows, eight in the ketotic group and eight in the healthy group, were used. All the cows were multiparous and in early lactation. A cow was defined as ketotic if she had the clinical signs, and positive results from Rothera and deepstick tests (Combur 9®).

Blood sampling and serum analysis:

Blood samples were taken from the jugular vein just before the liver biopsies were obtained (10). Serum was analysed for glucose, triglyceride, cholesterol, urea, total bilirubin, direct bilirubin, total protein, albumin, alkalen phosphatase, Aspartate amino transferase (AST), Alanine aminotransferase (ALT), Creatine phosphokinase (CPK), and Gamma glutamyltransferase (GGT) with a Technicon RA-XT autoanalyser. HDL and LDL-cholesterol (high and low density lipoprotein) levels were determined with commercial test kits (Boehringer Mannheim GmbH Diagnostica).

Liver Biopsy:

Liver samples were put in Baker's formol-Ca solution and fixed for 16 hours (11). Thin sections (12 μ) were cut from each sample and stained with oil Red O and Sudan Black B (12). Fields from each of five randomly selected blocks were examined under light microscopy and changes recorded (13). Cows with less than 10 percent fat in the liver were classified as mildy fatty on the basis of histological analysis of liver biopsy samples. Those with 10 to 20 per cent fat were considered moderately fatty, while those with over 20 per cent fat were considered severely fatty (14).

Student's test was used to analyse the data (15).

Results

Clinical findings:

The cows with primary ketosis had a history a gradual decrease in appetite and milk production, and rapid deterioration of body condition. They had firm, dry faeces. All the cows exhibited ketonuria.

Clinical chemistry:

The cholesterol and protein concentrations were significantly lower in the ketotic cows ($p<0.05$) than controls. The glucose and HDL-cholesterol levels were significantly lower in ketotic cows than controls. The chemical parameters are given in Table 1.

Histopathology:

The percentage of fatty infiltration of the liver parenchyma varied from 27 to 42 percent in seven ketotic cows and was 13 percent in one ketotic cow Figures 1-3).

Table 1. Clinical-chemical parameters and serum lipoproteins in all groups of cows.

Parameters	Ketotic group	Healthy group	T-test
Glucose (mg/dl)	31	47	9.407**
Triglyceride (mg/dl)	13.3	13.5	0.121
Cholesterol (mg/dl)	126.7	162.1	2.359**
HDL (mg/dl)	54.3	102.4	6.015**
LDL (mg/dl)	74.2	55.8	1.679
VLDL (mg/dl)	1.2	1.9	0.122
Protein (g/dl)	7.2	8.4	3.292*
Albumin (g/dl)	3.3	2.1	7.907**
Urea (mg/dl)	46.6	28.5	1.539
Total biluribin (mg/dl)	0.74	0.14	3.984*
Direct biluribin (mg/dl)	0.24	0.05	4.029**
AST (U/L)	121	132.4	0.392
ALT (U/L)	47.6	66.8	2.252
CPK (U/L)	661.8	329.6	2.704*
GGT (U/L)	18.9	19	0.081

* $p<0.05$
** $p<0.01$

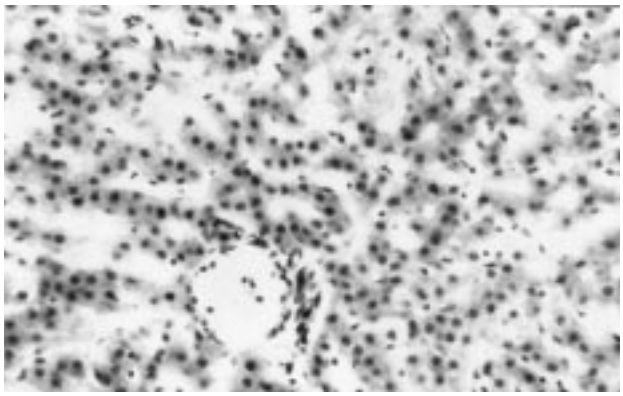

Figure 1. Microscopic appearance of the liver taken as a biopsy material from a healthy cow. Oil Red O, x190.

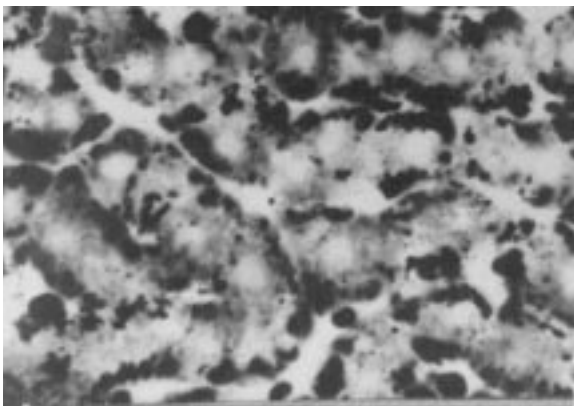

Figure 2. Microscopic appearance of the liver of a cow with moderate fatty liver syndrome. Sudan Black, x460.

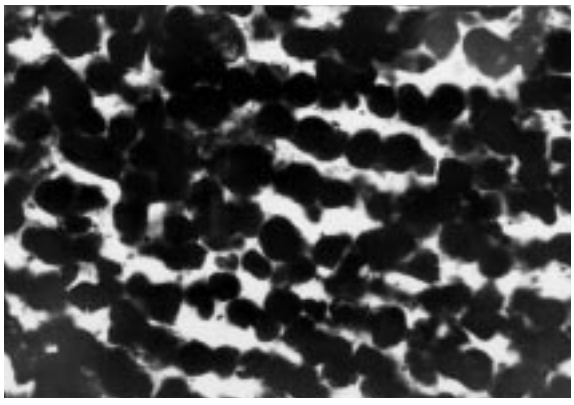

Figure 3. Microscopic appearance of the liver of a cow with severe fatty liver syndrome. Sudan Black, x280.

Discussion

There are 3 elements to be considered in the diagnosis of fat cow syndrome with sublinical fatty liver: history, clinical pathology and liver biopsy result. In cases of suspected subclinical fatty liver, diagnosis may only be possible by carrying out liver biopsy sample examination (4).

Classical clinical signs of spontaneous ketosis are loss of appetite, decrease in milk production, and rapid deterioration of body condition. All cows with fat cow syndrome show the typical signs of anorexia, depression, weakness, a marked decrease in milk production, ketonuira and progressive debilitation (5, 16-18). The clinical signs in the present study were also consistent with these. There is no significant alteration in the serum glucose of ketotic cows (18, 19). However, hyperketonemia and hypoglicemia are more obvious biochemical parameters (6, 16). The plasma glucose concentration is a more reliable guide to the extent of hepatic damage than total keton bodies (16). Blood glucose is below 40 mg/dl accompanied by ketonemia and ketonuria in many cows due to ketosis (18). In the present study, serum glucose concentration was significantly lower in the ketotic cows compared with controls. Cholesterol and phospholipids are the principal components of lipoproteins and accompanied by much smaller quantities of triglycerides. When there are disturbances in the lipoprotein synthesis, triglycerids accumulate in the liver cells with fatty liver as a result. A high proportion of the plasma lipids is transported in HDL. In cows with ketosis, the amount of cholesterol and phospholipids differed very little from normal cows, and the cows with fatty liwer had lower amounts of cholesterol and phospholipids (20-22). In the present study, the cholesterol levels in ketotic cows were lower than controls. According to Rayssiguer et al. (9), the animals with moderate steatosis have significantly lower values of HDL than the control animals as shown by the decrease in HDL-cholesterol and HDL-phospholipid concentrations, whereas the LDL fraction is not modified. Severe steatosis was associated with a large decrease in LDL concentration and a smaller change in HDL concentration. Therefore the determination of LDL cholesterol and LDL phospholipids might be a useful diagnostic aim. In the present study, the ketotic cows had lower amounts of HDL. These cows also had severe fatty liver. This difference between HDL and LDL levels in cows with severe fatty liver may orginate in the assessment of the percentage volume of visible fat in the hepatic parenchymal cells.

Hyperbilirubinemia is not necessarily indicative of liver dysfunction, and total and direct bilirubin levels should always be interpreted in association with other tests for liver damage (23). In contrast, bilirubin and bile acids are the most useful indicators of the alteration in hepatic uptake (8, 24, 25). Total bilirubin concentration increases in primary ketosis in cows (2). In the present study, there were also significant increases in total bilirubin ($p<0.05$) and direct bilirubin ($p<0.01$) concentrations in ketotic cows.

The plasma albumin concentrations fall significantly with the increase in severity of hepatic changes (16). In contrast, the albumin levels in the present study were significantly higher than in the control cows. The protein concentrations in ketotic cows were significantly lower compared with the control cows.

The elevation in CPK of ketotic cows in the present study may indicate damage to other tissues such as kidney and muscle as well as to the liver.

It has been reported that ketosis was associated with fatty liver (9, 10, 19). In the present study, seven cows had severe and one had moderate steatosis.

It was concluded that varying degrees of fat infiltration of the liver and some clinical-chemical parameters in all cattle with ketosis show that the disease is associated with fat cow syndrome. As a result, ketosis and fat cow syndrome should be considered together in diagnosis and treatment.

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