Liver Function in Dairy Cows with Fatty Liver

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SUMMARY

The purpose of this investigation was to establish any changes that may occur in liver function in dairy cattle with fatty liver. The liver fat content was determined in all cows histologically. Cows with fatty liver were grouped according to fatty liver fat content as cows with mild, moderate and severe hepatosteatosis. Some chemical parameters (bile acid, AST, ALT, GGT, ALP, CPK, glucose, total protein, albumin, globulin, total bilirubin and urea) were measured. There was a significant increase ($p<0.001$) in GGT, CPK and AST activities in cows with severe fatty liver than controls. The serum bile acid and urea concentrations were significantly higher ($p<0.001$) in cows with severe and moderate fatty liver than controls. The bile acid and albumin levels were also significantly different between severe and moderate fatty liver groups. In conclusion, bile acid, GGT, AST and albumin parameters seem to be helpful for liver function in cows with fatty liver. However, because of considerable variation of these results they should be interpreted with caution.

KEY-WORDS : liver function - fatty liver - dairy cows.

RÉSUMÉ

Modifications des paramètres biochimiques du foie stéatosé chez la vache laitière. Par M. SEVINC, A. BASOGLU, F.M. BIRDANE et M. BOYDAK.

Le but de cette recherche était d'établir les changements éventuels susceptibles d'apparaître parmi les paramètres de la fonction hépatique chez les vaches laitières atteintes de stéatose. Le degré de stéatose a été déterminé histologiquement. Les vaches ont été classées en faiblement, modérément et sévèrement stéatosées. Les paramètres biochimiques suivants : acides biliaires, AST, ALT, GGT, ALP, CPK, glucose, protéine totale, albumine, globuline, bilirubine totale et urée, ont été mesurés. Nous avons constaté une augmentation significative ($p<0,001$) en GGT, CPK, AST chez les vaches stéatosées par rapport à des animaux de contrôle. Il a existé par ailleurs une différence significative dans les teneurs en acides bilaires et albumine entre les groupes de vaches sévèrement et modérément stéatosées. En conclusion les acides biliaires, la GGT, l'AST, et l'albumine ont paru utiles à l'appréciation des modifications de la fonction hépatique des vaches stéatosées. Toutefois, en raison de l'importante variabilité de ces résultats, ces derniers doivent être interprétés avec précaution.

MOTS-CLÉS : paramètres biochimiques - foie gras - vache laitière.

Introduction

Fatty liver is a frequent disorder in high producing dairy cattle in early lactation [17]. The syndrome is characterized by progressive depression and failure to treatment. It is associated with excessive mobilization of fat to the liver in obese-well conditioned cows. This mobilization fat is induced by the negative energy balance that occurs during the parturient period [21, 28]. Affected cows usually have one or more of the following conditions: milk fever, ketosis, displaced abomasum, retained placenta, mastitis or metritis. Mild or moderate hepatic lipidosis may result in liver function that are not accompanied by hepatocyte destruction and increases in liver-specific serum enzyme activities [8, 13, 21]. Diagnosis of fatty infiltration of the liver in dairy cattle is presently based mostly on biochemical analysis, and biopsy and analysis of hepatic tissue [6, 27, 30]. Biochemical analysis had a relatively high negative predictive value but low positive predictive value with increasing severity of fatty infiltration [2]. The laboratory test for detecting liver dys-

function due to decreased functional hepatic mass or reduced hepatic blood flow are concentrations of endogenous substances normally extracted by the hepatobiliary system, eg, bile salts (bile acid) or bile pigments (biluribin) and plasma urea and total plasma protein; intravenous administration of certain exogenous dyes, eg, sulphobromphitalein (BSP) [11]. The tests above are discussed controversially. Because most of the test lack specifity, liver biopsy is frequently necessary in cases showing biochemical evidence of hepatobiliary disease.

The present study was designed to evaluate the liver function tests in dairy cattle with fatty liver.

Material and Methods

A) ANIMALS

44 cows with fatty liver and 12 healthy cows were used as a material. Cows with less than 10 per cent fat in the liver were classified as mildly 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 [4].

B) BLOOD SAMPLING AND SERUM ANALYSIS

Blood samples were taken from the jugular vein just before the liver biopsies were obtained. Separations of serum from coagulum were taken place immadiately within an hour by centrifugation for 15 minutes at 3000 rpm. Collected serum were stored at -20°C before analysis. Serum was analysed for bile acid, total bilirubin, direct biluribin, indirect biluribin, urea, total protein, albumin, globulin, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Gamma glutamyltransferase (GGT), Creatine kinase (CK) and Alkaline phosphatase (ALP). All analysis were performed on an automated analyser (Olympus AU 5200) using commercial test kits (Olympus Diagnostica GmbH). Total serum bile acid concentrations (SBA) were determined enzymatically (Sigma Chemically GmBH) on an semi-automated analyser (Technicon RA-XT).

C) LIVER BIOPSY

Liver biopsies were performed in the right, 11^{th} to 12^{th} intercostal space [25]. Liver samples were put in Baker's formaldehyde — Ca^{++} solution and fixed 16 hours [5]. Thin sections (12 µm) were cut from each sample and stained with oil Red O and Sudan Black B [18]. Fields from each of five randomly selected blocks were examined under light microscopy and changes recorded. The percent of fatty infiltration with less than 10 $\mu m^2/100\ \mu m^2$ were considered mildly fatty, and those with 10 μm^2 to 20 $\mu m^2/100\ \mu m^2$ moderately fatty while those with over 20 $\mu m^2/100\ \mu m^2$ severely fatty (18).

D) STATISTICAL ANALYSIS

To evaluate group differences the one way ANOVA test, and to determine significance of variation the Duncan test were performed (SPSS 7.5 for Windows) .

Results

Histological examination of liver biopsies in cows revealed in 17 cases mild, in 17 moderate and in 20 cows a severe fatty liver.

There was a significant increase ($p<0,001$) in GGT, CK and AST activities in cows with severe fatty liver than controls. The serum bile acid concentration was significantly higher ($p<0,001$), and the albumin concentration was significantly reduced ($p<0,001$) in cows with moderate and severe fatty liver than controls. The bile acid and albumin levels were also significantly different between in severe and moderate fatty groups. Although there were significant alterations in serum ALP, CK, T.bilirubin, urea and glucose activities, they were within normal range limits between fatty and control groups (Table I).

Discussion and Conclusion

The present study describes the biochemical changes which take place simultaneously in the liver and serum during the fatty liver and the correlation between them.

In various animal species (dog, horse and sheep) determination of total serum bile acid (SBA) concentration is considered to be a sensitive and specific indicator in assessment of liver function [3, 9, 14, 26, 30, 31]. However, in cattle the diagnostic value of the determination of SBA as a liver function test is discussed controversially: whereas some authors [1, 11, 20, 27, 28, 30] considered SBA as a reliable indicator for liver dysfunction, other investigators [10, 12, 15] doubt the diagnostic value of SBA. According to REHAGE *et al.* [16] the determination of SBA concentrations is of little value in the recognation of fatty liver or even liver failure due to the considerable variance of SBA concentration in dairy cows. In the present study, the SBA concentrations varied considerably between all group of cows (range: 10-170 µmol/l), whereas there were significant increases in SBA related to degree of fat infiltration.

Hypoalbuminemia is a common terminal feature of chronic liver disease, occurring when the functional hepatic mass has been reduced to 20 per cent or less [11]. The lowest albumin level has been found in cows with liver failure, but no in cows with fatty liver [16]. There is negative correlation in serum albumin between the degree of fatty changes and the albumin level in dairy cows [22]. In the present study, the serum albumin levels were significantly reduced in cows with moderate and severe fatty liver than control and mild fatty liver groups. The albumin level in cows with severe fatty liver was the lowest. However, there were significant alterations in serum urea and glucose activities in cows with fatty liver than controls, but these parameters were within normal range limits. So, albumin urea and glucose are indicative of the liver's synthetic function although these results must be interpreted with caution [28].

Measurement of serum activities of hepatic enzymes can be useful, but it also has limitations [8, 23, 24]. AST activity in serum is fairly well correlated to hepatic lipidosis, but this

Parameters	Control (n:12)	Fatty Liver			F	P
		Mild (n: 17)	Moderate (n:17)	Severe (n: 20)		
BileAcid (µmol/L)	34.9±8.3^{c}	51.5±8.2^{bc}	72.5±8.6^{b}	97.8±9.0^{a}	9.437	0.000
Glucose (mg/dl)	82.3±5.1^{a}	53.4±2.5^{b}	58.8±5.6^{b}	53.7±4.0^{b}	8.026	0.000
T. Proteine (g/dl)	7.9±0.1^{a}	8.1±0.2^{a}	7.1±0.2^{b}	7.7±0.2^{a}	4.775	0.005
Albumine (g/dl)	3.4±0.1^{a}	3.3±0.1^{a}	2.7±0.1^{b}	2.1±0.1^{c}	49.882	0.000
Globuline (g/dl)	4.5±0.1^{ab}	4.9±0.2^{a}	3.8±0.3^{b}	4.4±0.2^{ab}	3.986	0.012
T. bilirubin (mg/dl)	0.3±0.1^{b}	0.3±0.1^{b}	0.5±0.1^{ab}	0.6±0.1^{a}	4.393	0.007
D. biluribin (mg/dl)	0.1±0.3^{b}	0.1±0.3^{b}	0.1±0.3^{b}	0.2±0.3^{a}	6.131	0.001
I. biluribin (mg/dl)	0.2±0.1^{b}	0.2±0.1^{b}	0.3±0.1^{ab}	0.4±0.1^{a}	3.179	0.030
Urea (mg/dl)	18±1.2^{c}	19.4±1.3^{bc}	31.4±4.4^{a}	26.8±3^{ab}	4.445	0.007
ALP (U/L)	99.8±8.2^{a}	64.4±7.6^{b}	71.5±7.1^{b}	79.4±5^{b}	4.265	0.008
CK (U/L)	121.2±12.3^{b}	112.2±9.6^{b}	197.9±22^{ab}	228.4±4^{a}	3.996	0.011
GGT (U/L)	22.3±2.1^{b}	24.6±2.6^{b}	43.7±7.5^{b}	6.7±1.1^{a}	8.0.76	0.000
AST (U/L)	65.1±4^{b}	82.6±7.3^{b}	96.0±6.7^{b}	154.6±23^{a}	6.838	0.000
ALT (U/L)	29.1±1.7	24.9±1.3	27.0±1.6	29.3±1.5	1.823	0.152

Means with different supercrits within one row differ significantly ($p< 0.05$)

TABLE I. — Results of clinical chemistry ((s) in dairy cows with mild, moderate and severe fatty liver.

enzyme is nonspecific to hepatic tissue [17, 19]. GGT is more specific to liver tissue, but the correlation of these serum activities with hepatic lipidosis is not as high [7]. In the group of cows with liver failure, AST level was one of the highest parameters [16]. BOGIN *et al.* [8] found significantly the AST level in cows with severe fatty liver. Some of the increase of AST may have been due to muscle demage as evidenced by results of CK measurement [7]. In accordance with the references above, in the present study, the AST, GGT and CK levels were significantly higher ($p<0,001$) in cows with severe fatty liver. Although there was significant increase in CK activity in cows with severe fatty liver, it was within normal range limits. So, these AST and GGT increases may be due to severe fatty infiltration.

In conclusion, bile acid, GGT, AST and albumin concentrations seem to be helpful for liver function in cows with fatty liver. However, because of considerable individual variations of these results, they should be interpreted with caution.

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