

Non-invasive Diagnosis of Fatty Liver and Degree of Fatty Liver in Dairy Cows by Digital Analyses of Hepatic Ultrasonograms

A.S. Leaflet R1905

Gerd Bobe¹, Research Associate, Viren R. Amin, Assistant Professor in Electrical and Computer Engineering, Donald C. Beitz, Distinguished Professor of Agriculture, and Jerry W. Young², Professor Emeritus

Summary and Implications

The data demonstrate that digital analyses of liver ultrasonograms could diagnose fatty liver and degree of fatty liver (healthy liver, moderate fatty liver, and severe fatty liver with their ranges of 0 – 8, 8 - 12, >12% lipids of liver wet weight) with an accuracy of over 90%. Total lipid concentrations could be predicted for liver samples <8% of liver wet weight within 2% of wet weight. Therefore, ultrasound imaging is a reliable, non-invasive technique for determining liver lipid content and for diagnosing fatty liver in early lactation dairy cows to prevent loss of income for dairy farmers.

Introduction

Fatty liver (i.e., hepatic lipodosis) is associated with increased incidences of ketosis, mastitis, metritis (decreased resistance to disease), decreased and delayed conception rates, increased culling rates, and increased costs for raising necessary replacement heifers. It is estimated that up to 50% of the dairy cows in the United States go through moderate to severe fatty liver at or just after calving. For example, clinical and subclinical ketosis is estimated to cost more than \$300,000,000 yearly for U.S. dairy farmers. Fatty liver is characterized by an accumulation of triacylglycerol (fat) in the liver that impairs the function of the liver. The fat accumulation in the liver is caused by the increased mobilization of fatty acids from the adipose tissue when cows are in negative energy balance.

Liver biopsy followed by chemical analyses offers the only reliable diagnosis for fatty liver.

Ultrasonic imaging techniques provide noninvasive information about structures of tissues. The transmitted ultrasound waves interact with tissue constituents, particularly water, collagen, and fat and get absorbed,

reflected, or scattered. These interactions can be estimated from the reflected ultrasound signals and used to characterize tissues or organs of interest. Our goal was to develop a noninvasive, easy-to-apply, on-farm diagnostic test to determine the lipid content of the liver and to diagnose fatty liver in dairy cows to prevent metabolic diseases and decreased fertility.

Materials and Methods

Ultrasound images of livers (n = 49) were collected from 29 cows that were in the first 3 weeks of lactation. Cows were prepared for ultrasound scans by clipping the hair from a region on the right side from 10 to 90 cm below the spinous processes and from the 8th to the 13th rib. The cows were scanned with a commercially available real-time ultrasound system (Aloka 200V; Corometrics Inc., Wallingford, CT) with a 3.5-Mhz linear transducer (Aloka UST-5044-3.5). Vegetable oil was applied to the skin over the 10th, 11th, and 12th ribs to improve ultrasound conductance, and the transducer was placed on the oiled region. Sliding the transducer from the top to the bottom of the clipped area along the intercostal spaces (and parallel to the ribs) allowed ultrasound visualization of the liver from the top to the bottom margins. A liver image relatively free of blood vessels and other nonhepatic structures was digitally captured by using a portable computer with a frame-grabber hardware. The ultrasound image was preprocessed on a computer by selecting a rectangular region of interest with a homogeneous area of 128 by 128 image pixels. Then, the images were digitally transferred to a computer workstation (DEC station 5000; Digital Equipment, Manard, MA) and processed by using texture analysis algorithms. A total of 18 texture parameters was calculated from each region of interest. Following ultrasound scanning, a liver tissue sample was collected by needle biopsy from the location where the image was captured. These samples were analyzed chemically for dry matter and wet weight concentrations of total lipids and triacylglycerol. Statistical analyses were performed by correlation, discriminant, and regression analysis by using SAS.

Results and Discussion

The total lipids concentrations of the 49 liver samples covered a broad range of concentrations from 2.4 - 26.6% total lipids (Figure 1). The average was 9.2% total lipids, and the standard deviation was 6.2%. Based on previous research with fatty liver, we divided the samples into healthy liver and fatty liver samples by using 8% total lipids as the break point. By using discriminant analysis for

¹ Current address: Department of Food Science and Human Nutrition, Michigan State University, 3 GM Trout Building, East Lansing, MI 48824.

² Current address: 73 Alton Park Lane, Franklin, TN 37069.

classifying samples into one of the two groups, a linear equation of 18 ultrasound parameters was developed that classified more than 90% of samples into the correct groups. Only one of the 28 healthy liver samples and 2 of the 21 fatty liver samples were misclassified.

The fatty liver group was further subdivided into moderate and severe fatty liver by using 12% total lipids as the break point. By using discriminant analysis for classifying samples into one of the two groups, a linear equation of 18 ultrasound parameters was developed that classified 17 of the 21 samples into the correct group.

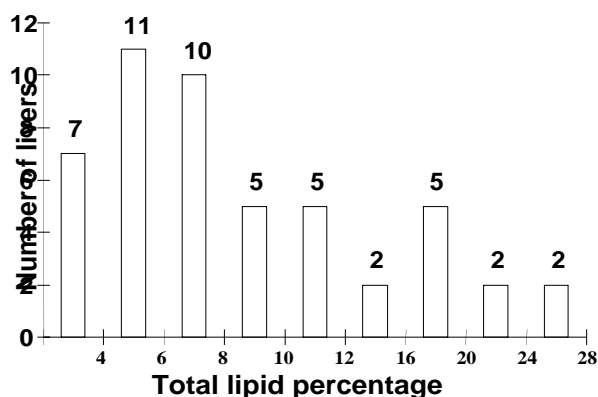


Figure 1. Distribution of lipid percentage (as % of liver wet weight) in liver biopsies from early lactation cows.

Correlation analysis showed that the association between ultrasound parameters and liver lipid percentage is not linear (Table 1). Therefore, we ran correlation analysis for the healthy liver and the fatty liver group separately. We found significant linear relationships between ultrasound parameters and total lipids in the healthy liver group with correlation coefficients around 0.6 in the healthy liver group, whereas texture parameters were not correlated with total lipids in the fatty liver group. We conclude that liver lipid percentage and ultrasound texture parameters have a linear relationship <8% total lipids; however, we could not detect a linear relationship between liver lipid percentage and ultrasound texture parameters >8%.

Table 1. Pearson correlation coefficients (significance levels in brackets) between liver lipid percentage and ultrasound parameters within the two liver health groups (n=49).

Ultrasound parameter	Healthy liver (n=28)	Fatty liver (n=21)
Fourier 1	- 0.65 (<0.01)	0.14 (0.55)
Fourier 7	- 0.63 (<0.01)	0.04 (0.88)
GMag1	- 0.37 (0.05)	0.07 (0.72)
GMag3	- 0.06 (0.75)	- 0.36 (0.11)
GMI3	- 0.14 (0.48)	0.08 (0.74)
GP2	- 0.33 (0.09)	- 0.24 (0.30)
HP1	- 0.65 (<0.01)	0.15 (0.51)
C135	- 0.28 (0.16)	0.11 (0.62)
R 090	- 0.36 (0.06)	0.09 (0.69)

Using only two parameters could predict total lipid concentrations of the 28 liver samples <8% of liver wet weight within 2% of wet weight.

Acknowledgments

The authors thank the management of the Iowa State University Dairy Teaching Herd for provision of cows, A. R. Hippen and P. She for obtaining liver biopsies and ultrasonograms, S. Deng for biochemical analyses of liver biopsy samples, and C. L. Hays, G. H. Rouse, and D. E. Wilson for assistance in digital analysis of ultrasonograms. The research was supported by USDA Grant number 93-37201-9343 and by Monsanto Co (St. Louis, MO)