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Diagnostic performance of cytology for assessment of hepatic lipid content in dairy cattle

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ABSTRACT

The objective of our study was to characterize the diagnostic performance of cytology for assessing hepatic lipid content (HLC) in dairy cows by comparing microscopic evaluation of lipid vacuolation in touch imprint slide preparations of liver biopsies with quantitative measurement of triglyceride concentration ([TG]; mg/mg of wet weight) in paired biopsy samples. Our study also sought to compare the diagnostic performance of liver cytology, plasma nonesterified fatty acid concentration ([NEFA]), and plasma β-hydroxybutyrate concentration ([BHB]) derived from a measurement performed on whole blood, for assessing HLC. Chemical extraction of TG from liver tissue remains the gold standard for quantifying HLC, largely because available blood tests, although useful for detecting some types of pathology, such as increased lipid mobilization, ketosis, or hepatocellular injury, are nonspecific as to etiology. Veterinary practitioners can sample bovine liver for cytological evaluation in a fast, minimally invasive, and inexpensive manner. Thus, if highly predictive of HLC, cytology would be a practical diagnostic tool for dairy veterinarians. In our study, liver biopsy samples from Holstein cows (219 samples from 105 cows: 52 from cows 2 to 20 d prepartum, 105 from cows 0 to 10 d in milk, 62 from cows 18 to 25 d in milk) were used to prepare cytology slides and to quantify [TG] using the Folch extraction method followed by the Hantzch condensation reaction and spectrophotometric measurement. An ordinal scale (0–4) based on amount of hepatocellular cytoplasm occupied by discrete clear vacuoles was used by 3 blinded, independent observers to rank HLC in Wright-Giemsa–stained slides. Interobserver agreement in cytology scoring was good. Corresponding plasma [NEFA] and [BHB] measurements were available for 187 and 195 of the 219 samples, respectively. Liver [TG] correlated more strongly with cytology score than with NEFA or BHB, and receiver operating characteristic curve analysis showed that cytology had better diagnostic performance than either NEFA or BHB for correctly categorizing [TG] at thresholds of 5, 10, and 15%. Hepatic lipidosis in high-producing dairy cows is of major clinical and economic importance, and this study demonstrates that cytology is an accurate means of assessing HLC. Additional work is indicated to evaluate the diagnostic utility of liver cytology.
Key words: β-hydroxybutyrate (BHB), cytology, lipidosis, liver, nonesterified fatty acids (NEFA)

INTRODUCTION

Hepatic lipidosis (**HL**, also known as fatty liver) is a clinical syndrome that occurs in dairy cattle with excessive accumulation of lipids, specifically triglycerides (**TG**), in hepatocytes. Clinical HL develops secondary to severe negative energy balance, most commonly in high-producing cows in early lactation, when mobilization of fat stores results in increased delivery of nonesterified fatty acids (**NEFA**) via the bloodstream to the liver, which is unable to oxidize or export TG in lipoproteins at an equivalent rate (Herdt, 1988; Bobe et al., 2004). Subclinical forms of HL occur, and some degree of TG accumulation in the liver occurs in most postpartum dairy cows. Although some degree of increased hepatic TG is not necessarily pathologic, the syndrome of HL is characterized by excessive TG accumulation and clinical illness of varying severity, from common postpartum diseases and poor response to therapy (Herdt, 1988). Clinical abnormalities associated with HL in dairy cattle—including metritis, mastitis, ketosis, abomasal displacement, and decreased reproductive performance—result in decreased productivity

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and major economic losses; annual costs of fatty liver in dairy cows in the United States have been estimated to be more than $60 million (Bobe et al., 2004; McArt et al., 2012).

At present, there is no reliably accurate blood test, or combination of tests, to quantify hepatic lipid content (**HLC**) or diagnose HL in cattle (Herdt, 1988; Cebra et al., 1997; Kalaitzakis et al., 2007). Hepatic lipid content can be measured definitively by chemical methods and can be estimated in various ways, such as histologically using lipid-soluble stains (Gaal et al., 1983), by flotation of biopsy specimens in copper sulfate solutions of different specific gravities, or ultrasonographically (Bobe et al., 2008; Starke et al., 2010; Haudum et al., 2011). In addition, papers dating back more than 50 years describe the use of fine-needle biopsy (**FNB**) and cytologic examination to detect hepatic lipid accumulation in cattle (Holtenius, 1961; Holtenius et al., 1962; Hoff and Cote, 1996). More recently, a study conducted by part of our research team using fresh bovine liver samples obtained at slaughter found moderate correlation between rank scoring of lipid vacuolation cytologically and histologically (Ríos et al., 2010, 2013). Lipid content was estimated to be higher by cytologic evaluation than by histologic evaluation, suggesting that cytology might be the more sensitive of the 2 methods for assessing HL. Those findings led us to hypothesize that microscopic assessment of lipid accumulation in cytological preparations accurately predicts liver TG content in dairy cows.

The main objective of our study was to characterize the diagnostic performance of cytology for assessing HLC in dairy cattle, using TG concentration ([TG]; mg/mg of wet weight) in paired liver samples as the reference method. Our study also sought to compare diagnostic performance of cytology with that of plasma [NEFA] and [BHB]. If shown to be a valid means of assessing HLC in cattle, FNB cytology could be a valuable part of the routine diagnostic repertoire of the practicing dairy veterinarian.

MATERIALS AND METHODS

Sample Acquisition

Liver biopsies and blood samples were obtained from multiparous Holstein cows (n = 105) enrolled in 2 separate studies conducted at the Cornell University Research Center (Ithaca, NY). Cows were between 20 d prepartum and 28 DIM. Tissue and blood was collected at between 1 and 3 time points per cow, corresponding to 20 to 2 d prepartum, 0 to 10 DIM, and 18 to 25 DIM. All samples were obtained following guidelines and protocols approved by the Cornell University Institutional Animal Care and Use Committee (protocols 2013-0064 and 2015-0097). Cows were kept in sawdust-bedded tiestalls with ad libitum access to food and water. Cows were exercised 3 times per week during the dry period.

Liver biopsies were obtained by 1 of 2 clinical veterinarians via percutaneous trocar approach that incorporates an O-ring near the base of the cannula (Hughes, 1962; Veenhuizen et al., 1991). Hair was clipped from the 9th intercostal space to the center of the paralumbar fossa, and the clipped area was washed with iodine soap and dried with paper towels. The biopsy site in the right 11th intercostal space was chosen after confirmation of correct placement with ultrasonography using a 7.5-MHz linear-array transducer (Ibex Pro, E.I. Medical Imaging, Loveland, CO) and 70% ethanol (Vet One, Boise, ID) as a coupling agent to visualize the area where the liver was found immediately adjacent to the peritoneum. Following surgical preparation of the site, the overlying skin and underlying intercostal muscle tissue was infiltrated with 10 mL of a 2% lidocaine solution (lidocaine 2% HCl; Vet One). An approximately 1-cm skin incision at the 11th intercostal space was made using a #22 scalpel blade to place the stainless steel trocar (31 cm long and 7.5 mm in diameter, Figure 1) into the abdominal cavity, directing the point of the trocar toward the left elbow. Tissue samples were placed on a 7.62- × 7.62-cm sterile nonwoven sponge to remove excess blood and used immediately to prepare cytology slides or snap-frozen in liquid nitrogen and stored at −80°C until later measurement of [TG]. The incision was closed with a disposable skin stapler (3M Precise, St. Paul, MN) and coated with aluminum spray bandage. Samples were successfully collected at each attempt.

Blood samples were collected from the coccygeal vessels using a 20-gauge, 2.54-cm needle and blood collection tubes containing sodium heparin (158 USP units; Becton, Dickinson and Co., Franklin Lakes, NJ). Plasma was separated within 1 h by centrifugation at 3,000 × *g* for 20 min at 4°C and stored at −20°C until further analysis.

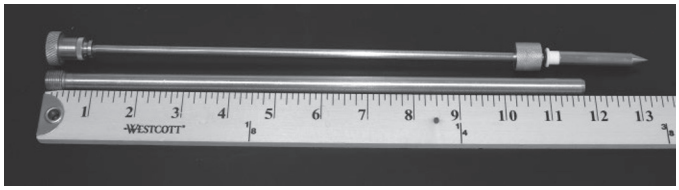

Figure 1. Photograph of the trocar device used to obtain biopsy samples. Color version available online.