Cowside Tests for Monitoring Metabolic Disease

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Abstract

The transition dairy cow is an animal at risk for the development of metabolic diseases. When necessary, veterinarians have traditionally relied upon analysis of blood samples, either in-house or at regional diagnostic laboratories, to aid in the diagnosis of these diseases. The development of routine herd monitoring has increased the amount of recommended blood sample analysis. An obstacle for doing more monitoring of fresh cows has been the difficulty and cost of sampling. Recently, cheaper and simpler "cowside" tests have become increasingly available for both the diagnosis of individual sick cows and the routine monitoring of fresh cow groups. Many of these tests have focused on the detection of ketosis. Proper understanding of the advantages and limitations of these cowside tests will aid in the selection of the proper test for the proper situation, thereby delivering the most useful results possible.

Introduction

The transition cow undergoes dramatic physiologic, metabolic, and social change in a relatively short period of time. Due to these immense changes, she is placed at an increased risk for the development of metabolic diseases, such as hepatic lipidosis, ketosis, and displaced abomasum. Dairy farmers have traditionally relied on veterinarians to aid in the diagnosis and treatment of these diseases. The diagnostics for these conditions have relied upon physical exam findings, and when needed, clinical analysis of blood samples, either in a local veterinary clinic or a regional diagnostic laboratory. As dairy production medicine has developed, increased emphasis has been placed on routine monitoring of cow groups, especially the transition cows. An important part of this monitoring is the analysis of blood samples, looking for evidence of metabolic disease. While much of this blood analysis has been done in diagnostic laboratories, it is becoming increasingly possible to perform some of this analysis at the farm or even "cowside". This presentation will provide more in-depth information on some of the relatively cheaper and simpler tests now available to do cowside diagnostics for both individual and herd-level medicine. It is not all inclusive but will cover the most common tests encountered in the field.

Tests for Ketones

Some of the most commonly available cowside tests are those for the diagnosis of ketosis. These tests may be used to confirm the diagnosis of ketosis in an individual sick cow, identify a cow with subclinical ketosis in a group of fresh cows for possible treatment, or be used to screen groups of cows to discover herd incidence rates of subclinical ketosis. The "gold standard" test for the diagnosis of subclinical ketosis, and the standard to which these tests are compared, is serum β-hydroxybutyrate (**BHBA**) measured in a diagnostic laboratory (Tyoppen and Kauppinen, 1980). The

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most commonly used level of serum BHBA to identify ketosis is a concentration ≥1400 µmol/L (14.4 mg/dL) (Oetzel, 2004). The cowside tests commonly used on dairy farms measure either acetocetate (**AcAc**) or BHBA and test urine, milk, or blood for the concentrations of these ketones. These tests are summarized in Table 1 and described in greater detail below.

Cowside tests for acetoacetate

Seemingly, the most common cowside tests measure levels of AcAc. Ketostix test strips (Bayer Corp. Diagnostics Division, Tarrytown, NY) and other similar test strips indicate the level of AcAc in urine samples. The test strips may be used to semi-quantitatively measure AcAc based on the degree of color change observed on the strips as sodium nitroprusside on the strips reacts with AcAc in the urine. The color change observed on the strips after 15 seconds is compared to a color chart provided with the strips and used to provide an indication of the concentration of AcAc in the urine sample. The color that corresponds to 1470 µmol/L is also described as a "small" level of AcAc. Using this color, Ketostix were demonstrated to be 78% sensitive and 96% specific when compared to the "gold" standard of a serum level of 1400 µmol/L of BHBA (Carrier et al., 2004).

KetoCheck powder (Great States Animal Health, St. Joseph, MO) is another test available for measuring concentrations of AcAc. An advantage of this test that it can be used to measure AcAc concentrations in milk, bypassing the need for obtaining a urine sample. The powder in this test turns from white to shades of purple when in contact with AcAc. Although no color scale is provided with the test, the degree of purple discoloration may subjectively indicate the severity of ketosis and the relative concentration of AcAc in the sample, with darker purples associated with higher concentrations. When using only slight discoloration or a "trace" level of AcAc as the definition of a case of ketosis, KetoCheck powder was shown to be 41% sensitive and 99% specific for diagnosing subclinical cases of ketosis compared to the accepted gold standard previously described (Carrier et al., 2004).

Cowside tests for BHBA

More recently, cowside tests for ketosis have focused on measuring BHBA levels. One such test is the KetoTest (Sanwa Kagaku Kenkyusho Co. Ltd., Nagoya, Japan; distributed by ELANCO Animal Health, Greenfield, IN). This test measures BHBA in milk and consists of test strips on which a reagent converts BHBA in the milk sample to AcAc and causes a purple color change. Using a provided color scale, the strip may be used to semi-quantitatively measure BHBA concentrations in the milk sample based on the intensity of the color change observed on the test strip. When compared to the previously described standard, the Ketotest was demonstrated to be between 27% and 59% sensitive and 76% and 99% specific for the diagnosis of ketosis based on a color observed corresponding to 200 µmol/L of BHBA (Geishauser et al., 2000; Carrier et al., 2004). When the cutpoint for diagnosis was changed to a color corresponding to 100 µmol/L, the test demonstrated between 73% and 80% sensitivity and 76% and 96% specificity (Geishauser et al., 2000; Carrier et al., 2004). Recently a new test, the PortaBHB test (PortaCheck, Inc, Moorestown, NJ), has been made available for cowside diagnosis of ketosis. It consists of test strips similar to the KetoTest and also measures BHBA concentrations in milk samples. According to unpublished data, it demonstrated similar sensitivity and specificity to the KetoTest.

One final test that uses BHBA levels for the diagnosis of subclinical ketosis has garnered a significant amount of attention during the last several years. This test system is the Precision Xtra™ meter using Precision Xtra™ Blood Ketone test strips (Abbott Laboratories, Abbott Park, IL). Originally

designed for use by human Type I diabetics at risk for ketoacidosis to monitor blood BHBA concentrations, this test system has now been extended to ketosis diagnosis in cows. The small handheld meter measures the BHBA concentration in a drop of blood that has been applied to a test strip, thereby making this test slightly more difficult to perform. The accuracy of this test, however, is excellent. Three recent studies examining the potential of the Precision Xtra™ system for diagnosing ketosis provided similar reports of excellent accuracy (Caldwell and Martineau, 2007; Oetzel and McGuirk, 2008; Burke et al., 2008). When these studies were combined, the meter and strips were 91% sensitive and 94% specific for the diagnosis of ketosis (Oetzel and McGuirk, 2009). Additionally, when the Precision Xtra™ results were compared to laboratory BHBA results there was an R^2 of 0.94, demonstrating excellent agreement (Oetzel and McGuirk, 2009). While there is the drawback of blood collection, this test is an extremely good tool for the detection of ketosis.

So which test to use?

The selection of which test to use for the diagnosis of ketosis in dairy cows has previously been the subject of an excellent review (Oetzel, 2004). As is often the case, test selection depends on the question that is asked. The KetoCheck powder, due to its high specificity and ease of use, has some value for diagnosing individual cows. It could be used to possibly confirm a suspected case of clinical ketosis or identify truly subclinically ketotic cows. Unfortunately, it will miss identifying a substantial number of subclinically ketotic cows. If the goal is to identify as many subclinically ketotic cows as possible for treatment, such as during routine fresh cow monitoring, then the KetoStix, KetoTest, or the PortaBHB tests would most commonly be selected. These tests would detect approximately 75% of subclinical ketosis cases using the previously described cutpoints, while giving very few false positives that would be treated yet truly non-ketotic. The decision on which test to choose would be based on cost, availability, and sampling ease. The Precision Xtra™ meter could also be selected for this previous test role due to its excellent sensitivity and specificity, but because of the increased work needed to run the test, most producers would tend to choose one of the milk or urine test strips first. The true value of the Precision Xtra™ meter lies in its ability to be used for herd-based ketosis monitoring. An accepted protocol for monitoring early lactation cows for herd incidence of ketosis is to test 12 or more cows for blood BHBA concentrations. If more than 10% of the cows tested have blood BHBA concentrations $\geq$ 1400 µmol/L, the group is considered to have a ketosis problem (Oetzel, 2004). The Precision Xtra™ meter is almost as accurate as a diagnostic laboratory for providing these results but can do it faster and cheaper. The urine and milk test strips have been used for this monitoring, but their lower sensitivity and specificity have made them poor tests for these types of herd management programs.

NEFA

To monitor a herd's transition cow program, it is useful to measure blood concentrations of nonesterified fatty acids **(NEFA)**. Elevated NEFA concentrations prior to calving indicate a negative energy balance and an increased risk for fatty liver and metabolic diseases. Current recommendations for the monitoring of prefresh cows consist of obtaining blood samples from 12 or more cows 2 to 14 days prior to calving for analysis. If >10% of the animals sampled have NEFA concentrations $\geq$ 0.400 mEq/L, the group is considered to be experiencing excessive negative energy balance and body fat mobilization. The NEFA testing has most commonly been done by a number of very capable diagnostic laboratories, with the only drawbacks being the cost of testing and the logistics of obtaining and shipping samples for analysis. Because of these drawbacks, on-farm tests for NEFA would seem to be of interest. One such test was the DVM NEFA

test, which used spectrophotometric analysis for the determination of NEFA in plasma samples, but it is no longer available. With the proper technical expertise, a centrifuge, and a simple spectrophotometer, it is possible to measure NEFA on farm using readily available diagnostic test kits, such as the NEFA-C kit (Wako Chemicals, Richmond, VA); however, the number of samples needed to justify the equipment and training of an individual would normally preclude such testing capabilities to a very large farm or veterinary clinic.

Serum Electrolytes

The ability to measure serum concentrations of calcium, phosphorus, magnesium, and potassium would aid in the field diagnosis of the cause of weak or down cows. Unfortunately, no simple cowside diagnostic tests exist at this time, so such blood analysis has remained primarily "in-house" at veterinary clinics, although large farms could also justify the equipment to carry out such analysis. The more recent development of portable blood analysis equipment, such as the VetScan i-STAT 1 (Abaxis, Union City, CA) and the Quick Test II (Midland Bio Products, Boone, IA) is beginning to bring limited blood assays for electrolytes and other substances to the field for rapid diagnosis by veterinarians.

pH

A final common cowside diagnostic test is the determination of the pH of either urine or rumen contents. Urine pH monitoring of prefresh cows on an anionic salt diet is routinely recommended by nutritionists and veterinarians. Mean urinary pH level of 8 cows within a prefresh cow group > 7.0 indicates dietary acidification is not adequate (Oetzel, 2004). Simple pH paper can be used to monitor urine pH, but relatively cheap and accurate digital pH meters are easily obtained. The advantage of a pH meter over pH paper is that it removes subjective interpretation from monitoring, and it can be used for measuring the pH of fluid obtained by rumenocentesis for the diagnosis of sub acute rumen acidosis. **(SARA)**. Current recommendations for SARA analysis by rumenocentesis are that of 12 cows tested, less than 25% should have a rumen pH d" 5.5 (Oetzel, 2004).

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Table1. Comparison of commonly available cowside tests for the detection of ketosis.

Product	Detection Level	Sensitivity	Specificity	Cost
KetoCheck Powder(milk or urine)	Trace	41%	99%	$13.95/50 g (~$0.28/test)
KetoStix (urine)	Small	78%	96%	~$0.24/strip
KetoTest (milk)	200 µmol/L	27-59%	76-99%	~$2.00/strip
PortaBHB (milk)	200 µmol/L	75%	91%	~$1.75/strip
Precision Xtra (blood)	1400 µmol/L	91%	94%	Meter: ~$15 to 20 Ketone Test Strips: ~$1.30/strip