Veterinary Technician Sessions

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Herd-based Testing for the Preventive Medicine Practice

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Abstract

Strategies have been developed to diagnose and monitor key metabolic and nutritional diseases in dairy herds. Veterinary technicians can play a key role in gathering and managing the herd-based data needed. Tests that can be used include ruminal pH (for subacute ruminal acidosis), blood β-hydroxybutyrate (for subclinical ketosis), cowside ketone tests (for identifying individual cases of ketosis), blood non-esterified fatty acids (for evaluating energy balance on pre-fresh cows), blood calcium (for parturient hypocalcemia) and urinary pH (for monitoring acidification of pre-fresh cows to prevent hypocalcemia).

Introduction

Metabolic and nutritional diseases typically increase as milk production increases and as dairy herds become larger. These factors favor the use of rigorous, quantitative monitoring of metabolic and nutritional diseases whenever possible. This paper will focus on strategies for testing and monitoring three critical diseases in dairy herds–subacute ruminal acidosis (SARA) subclinical ketosis (SCK) and parturient hypocalcemia (clinical plus subclinical milk fever). Enough quantitative data about these diseases is available to allow for development of a herd-based testing scheme. Additionally, these three disorders are gateway conditions for other problems such as laminitis, displaced abomasum, impaired immune function, retained placenta, and cystic ovarian disease. Other metabolic diseases can be important problems in dairies (e.g., hypomagnesemia, udder edema, hypokalemia, etc), but these are less common disorders and there are limited published data available to permit the development of a testing scheme.[15]

Interpreting Test Results for Groups vs. Individual Cows

Interpretation of herd-based tests for metabolic and nutritional diseases is very different than interpreting laboratory results for metabolites from individual cows. Test results from individual cows are interpreted by comparing the laboratory result to a normal range established by the laboratory. Normal ranges are often derived by calculating a 95% confidence interval (or a similar statistic) of test results from clinically normal animals. This approach is useful for making decisions about individual sick cows, but is not useful for interpreting herd-based test results. Interpretation of herd-based test results requires an understanding of how the each test affects cow performance (regardless of whether they are within the normal range or not), a statistically-based approach to determining sub-sample sizes, and an emphasis on monitoring subclinical disease prevalence instead of clinical disease incidence.

Interpreting Herd Proportions vs. Herd Means

Herd test results for metabolic diseases can be interpreted as either the mean test result of the subgroup sampled, or as the proportion of animals above or below a certain cut-point within the sub-sample. If a test is associated with disease when it is either above or below a biological threshold (cut-point), then it should be evaluated as a proportional outcome. For example, ruminal pH ≤5.5 puts cows at risk for SARA, with subsequent rumenitis and other complications.[4] High ruminal pH values are not important *per se* in the herd evaluation, as any value over 5.5 is considered acceptable. Therefore, interpret the proportion of cows with ruminal pH below the cut-point and do not be concerned with the mean value of the group tested.

Subclinical ketosis in dairy herds can be monitored by testing for blood β-hydroxybutyrate (BHB). Subclinical ketosis is also a threshold disease, and cows are affected only when BHB concentrations are elevated. Lowering BHB below a threshold concentration is of little to no biological significance to the cow. Therefore, herd-based BHB test results are interpreted on a proportional basis, and the mean concentration for the group of cows tested is of no concern. Blood BHB concentration above 14.4 mg/dl (1400 μmol/L) is the most commonly used cut-point for subclinical ketosis. This cut-point is considerably higher than the upper end of the typical laboratory normal reference range for BHB in individual cows.

Non-esterified fatty acid (NEFA) concentrations in blood can be used to monitor energy balance in pre-fresh cows. Elevated NEFA conceration prior to calving indicates negative energy balance and suggests increased risk for DA, ketosis and other problems after calving.[1] Low NEFA concentrations are not biologically important. The threshold for NEFA in pre-fresh cows (two to 14 days before actual calving) is 0.400 mEq/L. In herd testing situations, we evaluate the proportion of cows tested above this cut-point and not the mean.

The incidence of parturient hypocalcemia (clinical plus subclinical milk fever) in a dairy herd is evaluated by measuring serum calcium concentration within 12 to 24 hours of calving. A cut-point of less than 8.0 mg/dl (2.0 mmol/l) total serum calcium has been used to define parturient hypocalcemia.[13] Blood calcium results from fresh cows are interpreted as the proportion of cows below the cut-point.

Tests for herd-based evaluations of metabolic and nutritional diseases also require well-defined alarm levels for the proportion of animals above (or below) the described cut-point. Because of normal biological variation, a few individual cows are expected to be above (or below) the biological threshold. Alarm levels are established from research results and/or clinical experience with these tests in herd settings. Table 1 lists suggested cut-points and alarm levels for ruminal pH, BHB and NEFA test results.

Urinary pH in pre-fresh cows fed anionic salts is a useful test for herds that are fed supplemental anions before calving to help prevent milk fever. Urinary pH is a marker of whether or not the feeding program is achieving the desired acidification. The biological threshold for urinary pH is not one-sided. Rather, there is an optimal range for urinary pH of about 6.5 to 7.0. Urinary pH values that are either above or below this optimal range have adverse consequences. Therefore, urinary pH is evaluated by the mean of the group of cows tested, and the proportion of cows with high or low urinary pH is not calculated.

Appropriate Sample Sizes for Herd-based Tests

Adequate sample sizes are essential in herd-based testing. We must have reasonable confidence that the results (either a proportion or a mean) truly represent the entire population of eligible cows within the herd. In herd settings, we do not need to sample as many cows as a researcher would sample in order to achieve a 95% confidence ($P < .05$) in the results. Rather, a 75% confidence interval is both acceptable and practical.

The suggested minimum sample size for herd-based tests with proportional outcomes is 12 cows. This minimum sample size gives reasonable confidence (75% or more) that the herd classification from the test results of 12 cows will correctly represent the true classification for the entire group. Figure 1 shows an interpretation guide for ruminal pH testing results based on a sample size of 12 cows. Herd-based tests interpreted as means have a lower minimum sample size. For example, as few as eight cows can be sampled for urinary pH testing.

Cows chosen to be sampled must come from the appropriate "eligible" or "at-risk" group within the herd. It is of no clinical value to test cows for a condition for which they have little risk. Table 2 lists the eligible groups for herd-based tests.

More cows than the minimum sample sizes can always be sampled, but value of sampling more cows has to be compared to the time and money required to

Table 1. Cut-points and alarm levels for herd-based metabolic and nutritional disease tests evaluated as proportions.

Test	Cut-point	Alarm level proportion	Associated risk
Ruminal pH	≤ 5.5	> 25%	Subacute ruminal acidosis
BHB	≥1400 mmol/L	> 10%	Subclinical ketosis
NEFA	≥0.400 mEq/L	> 10%	Pre-partum negative energy balance, fatty liver

BHB = blood β-hydroxybutyrate; NEFA = plasma non-esterified fatty acids.
Adapted from Oetzel GR: Monitoring and testing dairy herds for metabolic disease. *Vet Clin North Am Food Anim Pract* 20:651-674, 2004.

sample the cows. Sampling additional cows is suggested when the results of a proportional outcome are very close to the alarm level, or when herd test results are not supported by clinical signs observed in the herd.

It is a common misconception that minimum sample sizes are larger for larger herds and smaller for smaller herds. This is incorrect – herd size actually has an inconsequential influence on the necessary minimum sample size.

In smaller herds, it may be possible to test the entire eligible group and still not meet the minimum sample size. This can be particularly true for pre-fresh cow testing (urinary pH and NEFA). For example, there might only be four cows in the pre-fresh group eligible for testing. All four should be tested; however, the sample size is probably too small to yield conclusive results. Additional cows can be tested later, as they enter the eligible group. Group results can be interpreted after test results from about eight (for urinary pH) or 12 (for NEFA) test results have been accumulated. If cows are repeatedly tested for NEFA as they approach calving, only the last test result before actual calving for that cow should be interpreted. Multiple test results from the same cow should not be used to achieve minimum sample size goals.

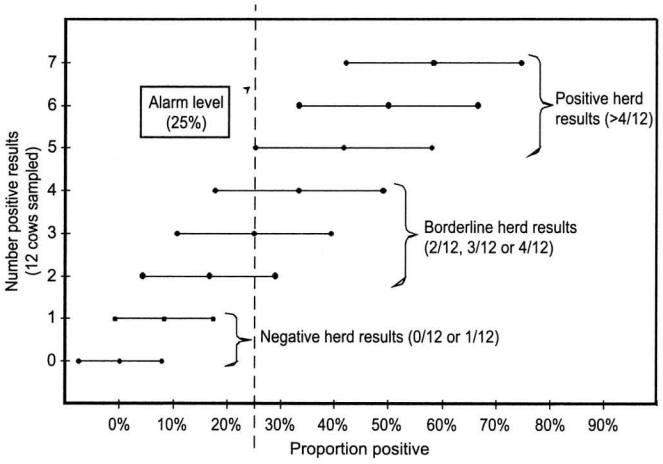

Figure 1. Interpretation of ruminal pH test results using 75% confidence intervals and an alarm level of 25% for test results from 12 cows sampled from within a group 100 cows. Adapted from Oetzel GR: Monitoring and testing dairy herds for metabolic disease. *Vet Clin North Am Food Anim Pract* 20:651-674, 2004.

Tests for Specific Metabolic and Nutritional Diseases

Subacute Ruminal Acidosis

SARA is diagnosed and prevented on a herd basis; there is no practical way to diagnose or treat in on an individual cow basis.[14] Clinical signs in dairy herds affected with SARA may include low or fluctuating dry matter intakes, low body condition scores, diarrhea, nosebleeds, unexplained deaths due to chronic inflammatory diseases, unexplained high cull rates due to vague health problems, milk fat depression and decreased milk production in the second and greater lactation cows relative to the first-lactation cows. None of these signs by themselves are diagnostic for SARA; however, considered together they form the basis for a presumptive herd diagnosis of SARA. It can be extremely useful to support a presumptive diagnosis of SARA in a herd with quantitative ruminal pH data.

Ruminal pH below about 5.5 for prolonged time periods is the apparent cause of the clinical signs observed in herds with SARA problems.[4] Evaluation of ruminal pH is challenging because it is difficult to obtain a sample for testing, and because ruminal pH varies from day to day within herds and time of day within cow. The methodology for collecting ruminal pH samples has been described in detail.[10,11]

A potential source of error in ruminal pH measurements is the calibration of the pH meter. A high-quality pH meter is recommended–pH paper is not sufficiently accurate and is influenced by the green color of the ruminal fluid. Field pH meters do not work well when operated at cold temperatures. It is best to conduct the

Table 2. Appropriate groups of cows eligible for different herd-based tests for metabolic and nutritional diseases.

Test	Eligible group
Ruminal pH	Lactating cows, about five to 150 days-in-milk (focus testing on cows five to 50 days-in-milk in component-fed herds and cows 50 to 150 days-in-milk in herds feeding a total mixed ration)
BHB	Lactating cows, about five to 50 days-in-milk
NEFA	Pre-fresh cows, ideally two to 14 days from actual calving
Urinary pH	Pre-fresh cows that have been on an anionic diet for >24 hours

BHB = blood β-hydroxybutyrate; NEFA = plasma non-esterified fatty acids.
Adapted from Oetzel GR: Monitoring and testing dairy herds for metabolic disease. *Vet Clin North Am Food Anim Pract* 20:651-674, 2004.

pH determinations in a warm milking parlor or office during cold weather. The ruminal fluid samples can be capped in their syringe (with the air excluded) prior to determining their pH. Also, pH electrodes may become dry between uses and lose accuracy; soaking the electrode in a buffer solution prior to calibration can prevent this. It is good practice to calibrate the meter twice (or more) before pH testing. After the last calibration, put the pH 7 and pH 4 buffers back on the meter to verify the correct calibrations.

The testing scheme for SARA works very well for herds with high (>30%) or low (<15%) prevalences of cows with low ruminal pH. It is not intended as a means of 'fine-tuning' diets for optimal ruminal pH–this would require much larger sample sizes and quite frequent testing. Herds with intermediate (16.7 to 33.3%) prevalences of low ruminal pH may require additional testing. Immediate dietary intervention is probably not critical in herds with intermediate prevalences, so it is not unreasonable to take some additional time to test more cows.

Ruminal pH sampling should be done around the time of the expected lowest point (nadir) in daily ruminal pH. In component-fed herds, the nadir in ruminal pH occurs about two to four hours after a grain feeding, and is probably the lowest after the last grain feeding of the day. In TMR-fed herds, the nadir in ruminal pH occurs about six to 10 hours after the first TMR feeding of the morning. Ruminal pH nadir occurs later in the day when dry matter intake is higher.

Subclinical Ketosis

It is difficult to assess the degree of SCK problems that a herd may be experiencing without doing herd testing. Clinical ketosis rates (as determined by dairy producers) have very limited value in assessing the true ketosis status of a herd. Producers have dramatically different definitions for clinical ketosis, and also have dramatically different abilities to detect ketotic cows. Producers in smaller herds tend to overestimate the incidence of clinical ketosis, and producers in larger herds tend to underestimate the incidence of clinical ketosis.

Herds with SCK problems in early lactation cows will typically have increased incidence of displaced abomasum and increased herd removals in the first 60 days-in-milk. High SCK herds may also have a higher proportion (>40%) of cows with milk fat to true protein percentages below 0.70 at first test after calving.[2] These clinical findings by themselves are not sufficient evidence to make a definitive diagnosis of a subclinical ketosis problem in a herd. Herd-based testing is required before a definitive diagnosis can be made.

The "gold standard" test for subclinical ketosis is blood BHB ≥14.4 mg/dl (1400 mmol/l). Clinical ketosis generally involves much higher levels of BHB (25 mg/dl or more). The alarm level for the proportion of cows above the cut-point of 14.4 mg/dl has not been well defined. Published research studies show an average SCK prevalence of about 15%, and I suggest using 10% as the alarm level for herd-based SCK testing. Figure 2 shows an example interpretation guide for BHB testing based on this alarm level.

As for SARA testing, the SCK testing strategy described here is designed to identify herds with either very high or very low prevalence of SCK. It is not intended to 'fine tune' or optimize a transition cow feeding and management program for SCK prevention.

The BHB test is performed on serum samples, and there are no special sample handling requirements. Blood samples for BHB testing should not be collected from the mammary vein. Mammary vein blood is lower in BHB because the udder extracts BHB during milk synthesis.[8] Blood BHB concentrations do exhibit post-feeding patterns and typically increase after feeding.[3,9] Sampling times should be consistent, and preferably about four to five hours after the first feeding of the day in order to capture peak BHB concentrations.[3] The post-feeding peak in serum BHB concentrations is caused by ruminal production of butyric acid. Surpluses of ruminal butyric acid (either from ruminal production or from silage) are mostly converted to BHB in the wall of the rumen.

A variety of cowside tests are available for ketosis testing of individual cows. However, no cowside test has perfect sensitivity and specificity compared to blood BHB. It is best to use the gold standard SCK test (blood BHB) for herd-level diagnosis and monitoring. Cowside

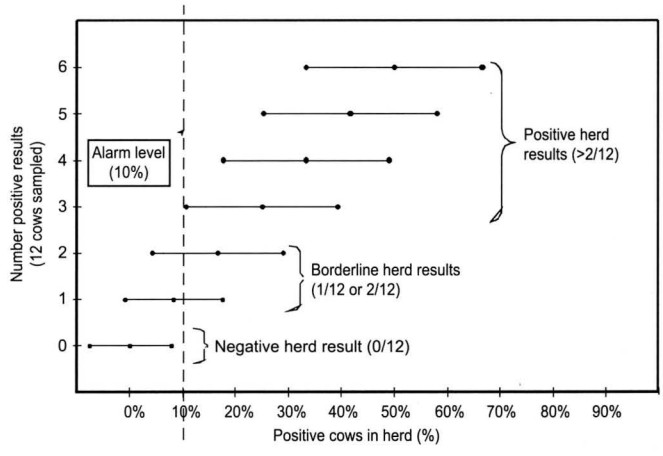

Figure 2. Interpretation of blood β-hydroxybutyrate test results using 75% confidence intervals and an alarm level of 10% for test results from 12 cows sampled from within a group of 50 cows. Adapted from Oetzel GR: Monitoring and testing dairy herds for metabolic disease. *Vet Clin North Am Food Anim Pract* 20:651-674, 2004.

ketosis tests have lower costs, require less labor and provide immediate results. This makes them useful for diagnosing clinical ketosis in individual, sick cows.

The blood NEFA test is used to evaluate energy balance prior to calving[6] Dry cows should be in positive energy balance up until the last 24 to 48 hours prior to calving. Negative energy balance is expected in milking cows, so blood NEFA concentrations are high after calving and can be difficult to evaluate. The SCK test of choice for post-fresh cows is blood BHB.

The NEFA test is best positioned as a secondary test in herds already known to have a high incidence of SCK. The NEFA testing helps determine whether the post-partum ketosis is caused by negative energy balance prior to calving.

The most commonly used cut-point for NEFA is ≥0.400 mEq/L in pre-fresh cows between two and 14 days from actual calving. NEFA concentrations normally rise in the 48 hours prior to calving, so results from cows that calve this soon after the sample was collected are difficult to interpret. They are usually discarded or interpreted with caution (values below 0.400 mEq/L are definitely negative, but higher values are not necessarily proof of a problem).

The alarm level for the proportion of cows with elevated NEFA concentrations within a group is not clearly known. I suggest using 10% as a reasonable alarm level. Because this is the same alarm level as for blood BHB in post-fresh cows (10%), the interpretation of NEFA results is the same as previously outlined for blood BHB (Figure 2).

The window of eligibility for NEFA testing is very small – only about 12 days, and you cannot know whether a cow will fit in the window until after she calves. In small dairy herds it may be difficult to sample enough pre-fresh cows to meet the minimum sample size required. Samples can be collected, frozen and later submitted as a group for NEFA analysis when actual calving dates are known and about twelve samples have been accumulated.

In large dairy herds, only a portion of the pre-fresh group needed may be sub-sampled for NEFA testing. In large pre-fresh groups, select cows that appear to be the closest to calving (based on due dates and visual observation), but avoid those cows in which calving appears to be imminent. In my experience, only about 75% of cows identified for NEFA testing using these criteria will actually calve two to 14 days later. Thus, expect to have to sample at least 16 cows in order to have 12 or more valid samples once actual calving dates are known.

Some pre-fresh cows may be in a maternity pen instead of the main pre-fresh pen(s). Do not avoid sampling cows in the maternity pen, as long as they do not appear to be imminently close to calving. Many of the cows in a maternity pen will not calve for several more days, and they are at very high risk for elevated NEFA concentrations because of the move to a new pen.

Concentrations of NEFA reach their nadir about four to five hours after the first feeding of the day[3] and peak just prior to the next major feeding. It is best to sample just prior to feeding in order to capture the peak NEFA value. It is acceptable to sample cows immediately after they have been locked up to new feed.

It is important to keep the plasma samples for NEFA testing cool or frozen from the time they are collected from the cow until the time they are received at the laboratory for analysis. At room temperatures some of the triglycerides normally present in blood may degrade to NEFA and falsely (but slightly) elevate the test results.

Herd Monitoring for Parturient Hypocalcemia

Both clinical milk fever and parturient hypocalcemia can be monitored in dairy herds. Limited data are available to assist in determining an alarm level for parturient hypocalcemia. Two studies with multiparous Holstein cows[7,16] record the incidence of both clinical milk fever and parturient hypocalcemia. In both studies, cows were fed control diets with and without anionic salts added. Feeding anionic salts reduced the incidence of clinical milk fever from 18.5% to 7.7% and the incidence of parturient hypocalcemia from 50.0% to 28.2%. I suggest alarm levels of ≥30% for parturient hypocalcemia and ≥8% for clinical milk fever in multiparous Holstein cows. Primiparous cows are at very low risk for low blood calcium around calving and probably should not be included in the monitoring program.

The best time to collect blood samples for monitoring hypocalcemia is about 12 to 24 hours after calving. In most situations the blood samples must be collected by on-farm personnel rather than by a veterinarian or technician. The farm then needs a means of separating the serum (or plasma) and storing it. Samples should be promptly picked up from the farm, processed and submitted to an analytical laboratory for calcium analysis.

Urinary pH for Monitoring Anion Dose

Dietary acidification by feeding supplemental anions is a means of reducing both clinical and subclinical hypocalcemia.[12] Urinary pH is a good monitor of systemic acidification and should be between 6.5 and 7.0. Urinary pH is interpreted as a mean value, and the suggested minimum sample size is eight cows. Testing should be done weekly, or even more frequently if urinary pH results are unstable. Urinary pH can be determined satisfactorily with pH paper – a calibrated pH meter is not required. On-farm personnel can conduct urinary pH testing (it is not technically difficult), but they actually tend to be very poor at doing this because

they are often busy with other, more urgent tasks. Having a veterinary technician check urinary pH values once a week helps assure that the task actually gets done.

The effect of time post-feeding on urinary pH is small when cows have access to feed throughout the day.[5] If feed access is not good throughout the day for pre-fresh cows, then the problem of inadequate feed availability is much more important than monitoring urinary pH.

Conclusions

Clinical impressions of metabolic and nutritional disease problems in dairy herds can be corroborated with herd-based metabolic testing. Ruminal pH with samples collected by rumenocentesis can be evaluated in herds that have clinical signs associated with SARA. Almost all dairy herds are good candidates to be tested for the prevalence of SCK via blood BHB test results. Consider NEFA testing of the pre-fresh cows to corroborate suspicions of pre-fresh negative energy balance in herds with SCK. And finally, monitoring cows on the day of calving for parturient hypocalcemia can provide early detection of diet-induced problems that lead to milk fever. If hypocalcemia problems are present despite supplementing the pre-fresh cows with supplemental anions, then it may be helpful to evaluate mean urinary pH of a group of the pre-fresh cows. Quantitative testing strategies based on statistical analyses have been used to establish minmum sample sizes and interpretation guidelines for all of these tests.

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